BRITISH JOURNAL
OF
OPHTHALMOLOGY

INCORPORATING
THE ROYAL LONDON OPHTHALMIC HOSPITAL REPORTS
THE OPHTHALMIC REVIEW AND THE OPHTHALMOSCOPE

VOL. 42 *AUGUST, 1958* No. 8

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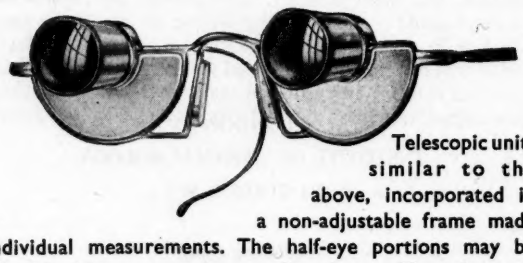

Telescopic units similar to the above, incorporated in a non-adjustable frame made to individual measurements. The half-eye portions may be glazed with either near or intermediate lenses. The telescopes may also carry astigmatic corrections.

Brit. J. Ophthal. (1958) **42**, 449.

COMMUNICATIONS

VISUAL DISTORTION IN AMBLYOPIA*

BY

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To assess accurately the vision of an amblyopic eye is in itself a problem. In an earlier investigation (Pugh, 1954), the recording of visual acuity in the amblyopic eye gave difficulty in every one of 150 patients. The usual test with a Snellen chart gave inconsistent results when tested on five or six consecutive charts during one examination. The patient's ability to read the letters appeared to alter with the shape of the letters, with the number of letters in the row, and also with the position of the letter in that row. These apparently erratic answers were given by good and less good observers alike. When visual acuity was very poor, this was understandable because the patient might be experiencing difficulty in holding a central fixation, but the same discrepancies occurred when the visual acuity had improved to 6/12 or better and central fixation appeared to be held without any difficulty. A detailed analysis of such inconsistencies has therefore been made.

Investigations

Method.—In this group, the patients' ages varied from 10 to 32 years. The visual acuity in the weaker eye at the beginning of this inquiry was 6/18, either having been already improved to this degree by treatment or being untreated in new patients. Thirty patients were selected for their intelligence and accuracy of observation, and because the visual acuity was such that targets subtending 20′ or smaller could be resolved (Polyak, 1941; Jones and Higgins, 1947).

Initial routine investigations included age at onset, amount and direction of deviation, retinal correspondence, binocular vision, and the refractive error of the eyes (Table, overleaf).

Patients with irregular astigmatism were excluded. The significant examinations for this investigation were monocular and concerned with analysing first the defect of the image of a letter as seen by the amblyopic eye, and secondly how this defect was influenced by such factors as the proximity of other letters, the position of the letter in a row, and the conditions of lighting.

* Received for publication August 16, 1957.

TABLE

PARTICULARS OF 30 SELECTED CASES

Visual Acuity of the Amblyopic Eye	None less than 6/18 at the beginning of the investigation	6/18
Refractive Error	Small error or none (less than 1 D sph. or cyl.)	2
	Hypermetropia	26
	Myopia (acquired)	2
	Astigmatism only (2)	
	Anisometropia (6)	
Direction of Heterotropia	Esotropia	11
	Esotropia with vertical factor	13
	Esotropia with vertical and tortional factor	5
	Mainly vertical and/or tortional	1
Degree of Deviation (with correction if required)	0 to 3°d	8
	4 to 6°d	16
	7 to 10°d	6

Apparatus.—The lighting installation consisted of a battery of eight 5-foot fluorescent "new warm" white 80 watt tubes suspended from the ceiling. This arrangement increased the amount of light on the test charts by 100 foot lamberts. When these top lights were turned off, the normal lighting of the Snellen type was 32 foot lamberts.

By this means the usual illumination on the Snellen type chart could be increased from 32 foot lamberts to 132 foot lamberts when the top lights were lit, the surround being at a luminance of 100 foot lamberts. The battery of lights could be put through a flashing mechanism which allowed varying speeds of flashing per second.

The usual roller type of Snellen chart was used in which three lines of the smaller letters can be shown at the same time through the opening in the metal frame. When required, this opening was covered with matt black paper which then served as a background for single letters which could be slotted into any position needed to correspond with the underlying chart. The advantage of this arrangement was that a series of single letters was shown and the answers recorded and these were then compared with the answers which were given either when a full line of the chart was visible, or when the same letters were seen under the same conditions of lighting and position but with additional letters either side and/or above and below. A scale was marked along the bottom edge to correspond with the centre area of the 6-metre tangent scale, and gave an approximate position of $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ degrees of movement as the eye looked along the line from right to left of centre. A slide of the tangent scale was also used in the orthoptoscope so that the exact angle of the direction of fixation could be noted as soon as the affected numbers appeared blurred.

Twelve letters were chosen: T F E H for the horizontal and vertical lines: X Z A N for the oblique lines with either horizontal or vertical lines; O C D U for the circular type of design.

The letters were printed in black, one on each white card 6″ square so that there were eight sets of twelve letters corresponding in size to each grading of the Snellen type chart from 6/60 size to 6/5 size. A second set with a white letter on a black

background was also used. Two other cards were used: a black circle on a white background and a white circle on a black background subtending 50′ at 6 metres.

Calibration of Light.—The lighting during the tests was varied in a definite sequence. The first recording was done with the standard 32 foot lamberts; the top lights were then put on and the vision again recorded, with the object of deciding whether the increase in the intensity of illumination would succeed in activating the more central parts of the fovea. Finally the top lights were flashed on and off at varying speeds and any further improvement was noted.

Procedure.—In all the tests the patient's dominant eye was first padded so that no light could enter and affect (by binocular light balance) the reaction of the amblyopic eye. When correcting lenses were needed, these were put into a trial frame in front of the amblyopic eye to ensure that they were correctly positioned. The trial frame also allowed varying sizes of pin-holes to be used, so that the tests would not be affected by any small amount of irregular astigmatism which might have escaped observation.

At each visit the visual acuity was taken on the Snellen chart in the usual way; it was then taken showing one line only and finally recorded when each letter of the same line was isolated by a large black mask with a hole in it. The difference between the acuity under these three conditions was noted.

The separate letter cards were kept in packs so that they could be shown easily and recordings were made showing which letters were faulted and how they were misnamed. The covering black paper was slipped over the Snellen chart and the separate letters were placed in turn in the central position on this black background. Thus each letter appeared black on a small white surround on a main black background, or white on a black background. The results were recorded, and as long as any letters were read correctly the next smallest series was put up and the visual acuity was noted until a point was reached at which no letter of that size could be read. In this way, at each visit, an accurate record was made of the letters most easily seen and also of recurring errors.

A detailed analysis of each letter was made. Each patient was asked whether all the component lines of the letter were equal in clearness of outline, blackness (or whiteness), and thickness. They were also asked to draw the letters exactly as they saw them to correlate with their verbal descriptions.

The patient's head was free but placed so that the amblyopic eye lined up approximately with the central point of the letter. While the patient fixed the central single letter, a second letter was placed to the side but within the width of the Snellen chart and therefore at an angle of $\frac{1}{2}$ or $\frac{3}{4}^\circ$ of abduction or adduction to the primary position. The effect of a second subsidiary image on the sharpness of the central letter was noted. Finally, the single letter was moved from one side to the other, and the patient turned his eye to follow the letter and reported any noticeable difference.

Results

The records of these patients showed several constant features:

(1) When a full Snellen chart was shown and, for instance, the 6/24 line

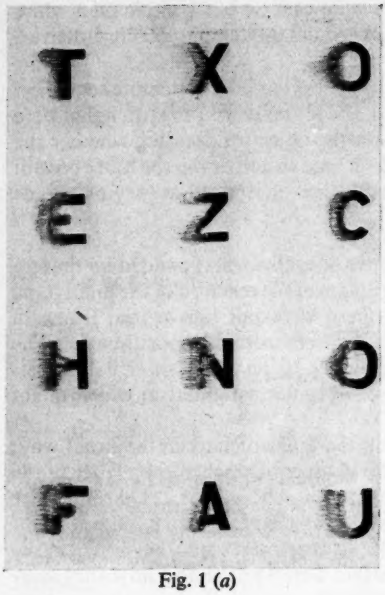

Fig. 1 (*a*)

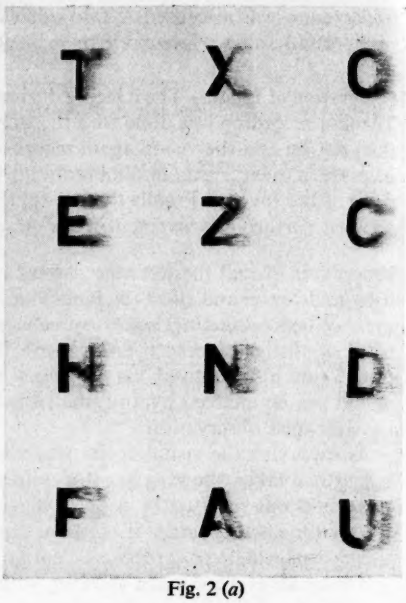

Fig. 2 (*a*)

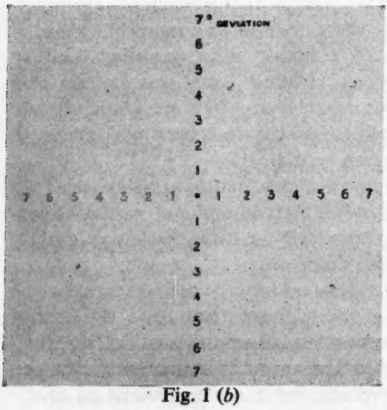

Fig. 1 (*b*)

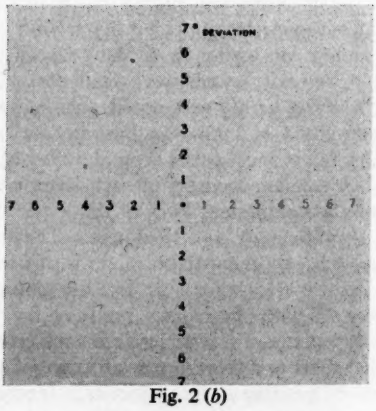

Fig. 2 (*b*)

was the smallest line read easily, the next line 6/18 was attempted with complaints that the central letters ran together defeating judgment. As soon as each letter was isolated with a black surround it became quite clear and steady and was read correctly, and usually the next line of isolated letters was read in part. In every case the patient read at least one more line as soon as the adjacent letters were covered. At the limit of resolution it was obvious that even when isolated, the shape of the letter made a difference, some being much more easily read than others.

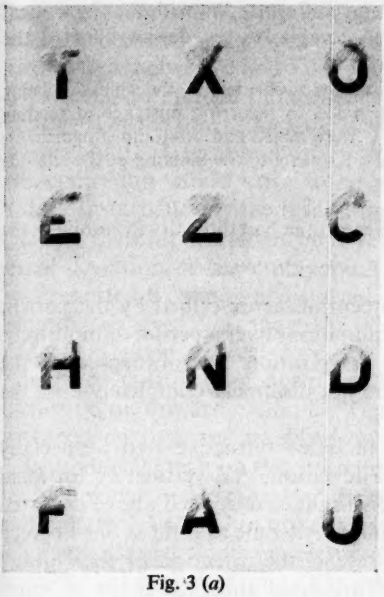

Fig. 3 (*a*)

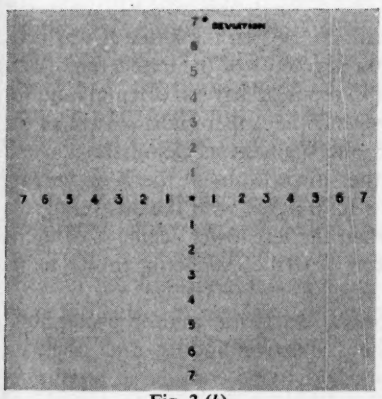

Fig. 3 (*b*)

(2) When separate letters were shown, it was found that certain letters favoured by the patient were sometimes read two or even three Snellen grades smaller than the initial test-line or the whole chart. A detailed analysis of each letter revealed that in every case the description showed that most of the letters were seen with a consistent distortion; consistent that is for each patient. At first the main difference between the descriptions given by different patients appeared to be that those with a left amblyopic eye appeared to see the pattern of the letter disturbed on the left side, while those with a right amblyopic eye had trouble with the right side of the letter, but this was often not so simple. Some patients found a pattern interference in the oblique lines, so that a part of one diagonal of an X might be much clearer than the other segments but the whole length of the oblique line was not affected as might be the case if the blurring were due to an astigmatic error of refraction. These variations led to the use of the round black circle on a white background as a key to help elucidate the areas of abnormality. Thus a patient with a left amblyopic eye might find it easy to explain that from 12 o'clock through 3 o'clock down to 6 o'clock the black circle had a good outline and was uniformly black in colour, whereas on the opposite side through 9 o'clock the outline became "jagged", "dragged", "smudged", or "rubbed", the blackness faded, and the circle appeared to be flattened. This would agree with the pattern distortion described for the individual letters: the vertical letter H would be seen with a clear black stroke on the right side, but the left upright would be described as thinner, with blurred edges, and greyer in colour. The letter T would be seen with a clear black edge on the right of the crossbar, while the left side would be rounded, blurred, and indefinite.

A left eye of a subject who had a small degree of esotropia, without any vertical deviation or large degree of astigmatism to complicate the design, showed the following errors when examined monocularly:

The pattern disturbance was similar through all the twelve letters, the right side being sharp, and the left side abnormal. This defect led to recurring mistakes in reading letters, the circular letters such as D O U and C were mixed and often indistinguishable from other letters; an X would look rather like a K owing to the blurring of the left side of the letter.

Conversely, the right eye of a subject with esotropia had difficulty in resolving the right side of the letters.

The position of the area of faulty perception as described by the patient remained constant throughout many examinations over a period of months or even years. Alterations occurred not of position but of degree as the visual acuity improved to normal, but did not disappear completely until the acuity was 6/6.

Factors which had to be considered included refractive error, especially if complicated with astigmatism, lateral deviation, and vertical or tortional deviation. The simple lateral pattern disturbance described above occurred in patients with a small degree of esotropia only (some as little as one degree). When a small vertical deviation was present, the direction of the "drag" might be shifted slightly up or down. This could affect the shape of letters with oblique lines such as X, shortening one segment and possibly making it look like a Y, and confusing an N with an H. When the letter X was considered not as two oblique lines but as four segments of the letter, the disturbance was usually described as occurring in separate halves, often giving, for example, the lower left corner as the best and the upper right corner as the worst. A number of patients described one segment as being shorter and running into the background, and the other three limbs of the X as varying in order of clearness; such a description would agree with the answers given on the circle card test. For instance, when the left lower segment of the X was defective, the circle would have been described as being faulty in its contour in the region of 7 o'clock.

When astigmatism and/or a small vertical deviation and/or cyclophoria were present, a correlation between the subjective finding and objective measurements was difficult. Single cases had to be considered because of the rarity of the combinations. Thus one girl aged 13 years had a vertical deviation of 3 to 4° following a naevus which developed on the right upper lid at the age of 2 months. Radiotherapy restored the lid to normal by the age of 5 years but the eye was amblyopic. The refraction of the eye had changed from a hypermetropic astigmatism to $\frac{-3{\cdot}25}{+3{\cdot}5}$ 90, and with this correction the visual acuity had developed from 6/36 to 6/9 in the last 3 years. Her description of letter shapes was consistent letter to letter and through the

stages of treatment the top left area of the circle between 10 and 12 o'clock was the poorest part of the pattern improving towards 2 o'clock, and the clearest was between 3 and 5 o'clock but fairly good right round to 10 o'clock. This seems to exclude the astigmatism as the single or even as the primary factor. On the other hand, the deviation was 0° variable, 2 to 4° right hyperphoria, and 5° excyclophoria. Without attempting detailed correlation, the connexion would seem to be a deviation downwards of the right eye with a disturbance of the resolution when the eye fixes upwards.

Thus there appeared to be a relationship between the direction of the usual deviation of the amblyopic eye and the direction of fixation which was reported as giving a distorted design. A right esotropic eye saw the pattern "dragged" on the right side and conversely with the left eye. An additional slight vertical deviation would complicate the direction of the "drag" to shift it up or down. A main vertical deviation downwards gave distortion on upward fixation. The astigmatic error had an effect, but this was secondary to, and modified by, the deviation.

In monocular tests on the orthoptoscope, the patient was asked to look at a tangent scale, to alter fixation from the left figure 1 to the right figure 1, and then to 2 3 4 5 6 7 in turn, and to compare the clearness of the right and left numbers, each figure representing one degree more adduction or abduction from the primary position. The results were the same as in the letter tests. The girl with the vertical deviation saw no difference when she changed her fixation from right to left but found that the figures above the horizontal were seen less well when fixed than the lower letters. In this test the actual amount of movement made as each number was fixed in turn was known because they were the numbers of the tangent scale. Further monocular tests with Chavasse slides showed differences which agreed with the other tests.

Since single letters were seen so much better than the Snellen chart lines, additional letters were placed at the side of the single letter to test whether this would have an effect on the acuity. This was found to be the case, the effect of the additional letters being to make the resolution of the first letter more difficult. Some detailed observations by a young science student aged 21 seem to be worth including.

His right eye had a refractive error of $\frac{+0{\cdot}5}{+1{\cdot}75}$ 60, and his visual acuity was 6/18 with or without correcting lenses. Binocularly, the amblyopic eye was 3° esotropic and 1° hypotropic. On analysis he described the usual difficulty in seeing the right side of the letters but with more careful observation than applied by most of the patients. For instance, each T H E and F was blacker and more definite on the left side, but, towards the right, definition faded away so that there was no clear end to the horizontal bars of the E F and the T, and the right-hand side of the H was less steady than the left. He explained that, whereas he could examine the left upright of the H from top to bottom easily and could see that the horizontal

bar was attached to it, the horizontal line faded towards the right and he found it very hard to keep his eye focused on the right vertical upright. The horizontal bar tended to appear lower and the letter was often confused with U.

When the oblique letters N X Z and A were shown to him separately, much the same effect was described, except it was complicated by the different axes of the oblique lines. He described the N, both the black letter on the white background and the white letter on the black background, as being in five grades of grey, the white letter being slightly easier to analyse; the left vertical line was very much better than the oblique line or the left vertical line. In the letter X he gave four grades of tones—again the two left sections were sharp, fading off toward the right. In the letter A he described one sharp left line which gradually faded through five tones to an indefinite smudgy right edge.

He found the circular letters O C D and U the most difficult of all to identify. The poor right edge of the O prevented him from distinguishing the O from the C. The U gave less difficulty, but again had a very smudgy right-hand vertical.

The vision was recorded on many visits during 8 months under the same conditions, and the patient's answers were consistent during each session and on each visit. Disks with small pin-hole apertures were used to see if they made any alteration in the pattern and to exclude the possibility of the pattern's being due to irregular astigmatism. There was no alteration through the small holes, but the patient thought sometimes that they did help to "shut out the surround". They made no difference to his answers. The difference between the visual acuity assessment was that, whereas the patient could read the vertical letters when they were as small as the 6/9 Snellen type, the oblique letters needed to be of the 6/12 size and the circular letters were not differentiated until they were 6/18 size. A whole line shown at the same time was read at 6/18 with difficulty because the letters ran together. The patient described this confusion as due to an "iridescence" from the adjacent letters.

After 10 months of obsessional application in his spare time this young man could read the chart down to 6/9 and all the 6/6 line except the fifth and sixth letters out of the seven. On any chart the letters in this position ran together and were difficult to disengage. His head and eye were free to turn as he wished, but, in spite of this, he found the letters confused in this one particular direction of regard.

All patients were able to read about half to one line more when the extra light was employed but they tended to tire more quickly. A flashing apparatus was used with speeds varying from one to five flashes per second; this did have an effect but made the analysis of the letter shapes more difficult. The effect of flashing will be considered separately from this investigation.

Discussion

The foregoing results gave evidence that an amblyopic eye can resolve single letters much more easily than letters of the same size presented in a row. This effect is not found in non-amblyopic eyes, although it has been noted that certain shaped letters are normally seen with more irregularity than other shapes. Tscherning (1904) observed that some letters are easier

to read than others and thought that the legibility of a letter did not depend entirely on the size of the intervals separating the different lines. Hartridge and Owen (1922) agreed with this. The suggestion was that some retinal astigmatism was present and that some of the inconsistencies could be due to small astigmatic errors of refraction which could not be corrected by lenses. However this may be for the slight irregularities seen by a normal eye, the presence of any significant retinal astigmatism or defect of the refractive media as a cause for the distortion described by the patient using an amblyopic eye is disproved by the improvement and final correction of the condition by treatment.

The pattern disturbance, which the patient described as occurring when his amblyopic eye examined a certain region of a letter (this region being particular and constant for himself), was an asymmetrical distortion of the letter form. When the disturbance occurred in the significant part of the letter, the difficulty in naming the letter was greater than if the blurred pattern happened, for example, to the right-hand side of a C, where there would be an absence of contour. There was in all cases a consistency of this pattern distortion. Irregular astigmatism, which might have been the cause of an asymmetrical image, was excluded by pin-hole tests.

This distortion of pattern had been found previously to be a regular feature of the visual acuity of an amblyopic eye and was thought to be possibly due to lack of central fixation in an eye with a defective visual acuity. To exclude any such complication, these patients were chosen with visual acuity of at least 6/18 at the beginning of the investigation (many improving to 6/12–6/9–6/6 later). Since a 6/12–6/9 letter subtends 10 or 7·5 minutes of arc at the fovea, the receptors involved in resolving letters of this size must be the slender cones of the floor of the fovea centralis.

In normal subjects, Higgins and Stultz (1948) reported:

"A better visual acuity is obtained from measurements with a parallel-line test object when the lines are vertical or horizontal than when they pass diagonally through the visual fields."

The pattern disturbance described by these patients was always asymmetrical whether the letter was composed of vertical and horizontal lines, diagonal or curved lines.

The problem is that an eye with good central fixation, when moved freely to fix all parts of a letter, can resolve one side of the letter easily but has difficulty and a sensation of frustrated fixation on the other side. The abnormal appearance of this side of the letter was described variously, the visual effect being apparently outside the usual visual experience of the normal eye; descriptive terms such as "iridescent", "dragged", "unreal", "splintered", even "not in the same plane", suggested the same quality of pattern disturbance. The effect described was quite unlike that of suppression, the letter sometimes appearing to have rather more to it than less. Several descriptions

suggested a doubling of one section of the letter, the doubled part being ghostly and pale (no irregular astigmatism was present and the doubling subsequently disappeared with treatment). There is no explanation in the theory of the optics of the formation of the image to account for these effects so described, or for the directional preference in resolution of one side of a letter as opposed to the other side.

If a single letter is the target object and a second letter is placed to the side of the distorted outline, some increase of disturbance occurs, but if the second letter is placed to the side of directional preference, the target letter becomes much more confused in outline and, when a full Snellen chart line is visible of a size letter which is easily read singly, considerable confusion of letters occurs. This was explained by patients as being due to the letters running together; the *mesial* letter (for that eye) appeared discrete and steady but, as the eye fixed the subsequent letters, these appeared to run together "as if a fold occurs to run one letter into the other". Since each letter became clear and was read correctly when isolated, the confusion must have been due to the presence of the extra letters in the row. Many good observers explained that, although they could see one end letter easily, they could not disengage the following letters one from the other and had difficulty in counting how many of them there were in that direction.

A subject with heterotropia will have one eye which, under ordinary conditions, is turned (however slightly) so that the optical system and pupil of the eye receive the incident light obliquely as the dominant eye fixes its target object. In considering what difference this may make to the response to the light stimulus by the foveal receptors of such an eye, the observations of Stiles and Crawford (1933) may have significance. To recapitulate, they demonstrated that "rays entering the pupil at points distant from the axis are not so effective visually as rays entering along or near the axis", also that "pencils reach the retina with approximately equal intensities but the brightness-producing efficiency of rays incident on the retina varies with their angle of incidence"; they suggested "that the variation of luminous efficiency is probably retinal rather than pre-retinal".

O'Brien (1950), working on local variations of the Stiles and Crawford effect, found a phenomenon which appeared to be due to localized tilting of groups of cones. O'Brien (1951) examined the relationship of the structure of the retina to the three factors influencing visual acuity (*viz.*, pupil size, luminance, and position of the image on the retina). He elucidated the optical mechanism whereby the spread of rays after focus (with consequent loss of image quality) is prevented. The optical processes within the cones were considered and demonstrated with large model cones and a suggested explanation of the Stiles and Crawford effect was made by these processes. When light enters axially into the larger end of a cone it does not escape before reaching the cylindrical outer segment and is then reflected down the outer segment to escape only at its outer end. Most light absorption occurs in the outer

segments of the cones. According to this theory of cone geometry, a ray entering not parallel to the axis will not be reflected totally down the cone and light will escape into the intercellular fluid.

This explanation of cone behaviour may supply some explanation of the behaviour of the cones of the fovea centralis of an amblyopic eye. If these receptors were in normal alignment, there would be no possibility of incident rays reaching neighbouring cones and spoiling the sharpness of the image. But the evidence is that several letters in a row appear to interfere one with another, an effect suggesting that the image of the target letter is being confused by secondary or ghost images cutting across and disturbing the primary image. This suggests that the receptors themselves are either tilted or disorientated, producing the effect that light rays entering the receptors parallel to their axes will have entered the optical system from a point slightly away from the line of the normal position of the visual axis. It is also possible that an oblique stimulus falling on the fovea through the years may have some effect locally on receptors designed to receive light stimuli axially into their outer segments. Although phototropic movements have not been shown to occur in man and the evidence is that they occur only to a small degree in mammals (for summary see Duke-Elder, 1932), it might be that under abnormal conditions some such changes could occur in man.

Summary

(1) The visual acuity of an amblyopic eye appears to be one, two, or three grades worse when tested on a whole line of a Snellen chart than when these letters are isolated or separate Snellen letters are shown.

(2) The difficulty a patient has in reading consecutive letters in a row appears to be due to an effect of one letter overlapping another and the patient's inability to disentangle one letter from another.

(3) The power to resolve single letters varies with the shape of the letter. This was found to be due to a distortion of outline which occurred when the patient fixed one side of the letter but was not present when he fixed the other side. When the area of pattern disturbance coincides with a special feature of the letter's shape, that letter is not resolved correctly.

(4) The direction of pattern disturbance is consistent and constant and appears to be directly related to the heterotropia which is present binocularly. This direction appears to be opposite to that of the heterotropia, but may be modified by a significant astigmatic error acting as a secondary factor.

(5) This directional effect can be corrected with treatment, thus proving that it is not due to irregular astigmatism or other organic irreversible conditions. The effect is quite unlike that due to suppression.

(6) The graduated single letters used subtend 10 to 7·5 minutes of arc at the fovea and therefore must be resolved by the slender cones on the floor of the fovea.

(7) There seems to be no accepted theory of vision to account for these effects, but if the constantly tilted position of the visual axis causes a tilting of the foveal receptors, an explanation can be suggested. The possibility of phototropic movements may possibly be considered.

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Brit. J. Ophthal. (1958) **42**, 461.

A REPORT ON THE ATTEMPTED ISOLATION OF THE VIRUS OF TRACHOMA*†

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THE consensus of opinion at the present time is that trachoma is a virus disease, being due to the agent first described by Halberstaedter and Prowazek (1907), who produced an experimental infection by inoculating the conjunctivae of apes with material from a human case of trachoma. Both the apes and the human cases from which the material came showed characteristic inclusions in the cytoplasm of conjunctival cells.

Support for the work of Halberstaedter and Prowazek came from the experiments of Thygeson (1934), Thygeson and Proctor (1938), and Thygeson and Richards (1939), who produced infection in monkeys and in man, and also made estimates of viral size by filtration. Unfortunately they were unable to find a simple means of viral cultivation and, as with other workers, the disease they produced in monkeys was unlike the human infection.

Poleff (1939) stated that he had been able to cultivate the virus in the fertile chick embryo, as did Macchiavello (1944), and Arakawa, Kitamura, Mitsui, and Tanaka (1953) claimed to have isolated a virus in mouse brain. None of these reports has been confirmed and Macchiavello's account of the experimental infection in a volunteer suggests that he was dealing with an adenovirus rather than trachoma.

There is general agreement that no animal other than the monkey can be infected by the conjunctival route, and even the monkey infection is irregular and not typical of the human disease. Furthermore, the identification of inclusions in monkey infections has been made in only a minority of experiments.

Several workers, such as Thygeson (1939), and Murray, Chang, Bell, Tarizzo, and Snyder (1957), have tried to isolate the virus in tissue culture, but have apparently failed to obtain a transmissible agent which will fulfil the criteria necessary for the identification of a new virus.

In the following report an account is given of various attempts made to isolate the virus in three different areas of the world, namely in Jordan and in Gambia where the disease is endemic and a large proportion of the population is infected, and in London, where occasional cases were seen in the outpatient departments of Moorfields Hospital. These latter patients were old cases who had been infected before arriving in England.

* Received for publication August 16, 1957.

† Since this paper was submitted for publication the successful isolation of strains of trachoma virus has been accomplished by T'ang Fei-Fan and his co-workers (1957) and by Collier and Sowa (1958). Strains of virus isolated are sensitive to penicillin which may account for the failure of isolation reported here.

Material

Material for isolation of the virus was taken from the conjunctiva of infected persons by means of a spatula made from aluminium wire (throat swab wire is adequate for this purpose). The wire is hammered flat until a spatula about 5 mm. in diameter is obtained; all the edges are carefully smoothed with an Arkansas stone and the scraper is sterilized by heat. To obtain conjunctival cells, the conjunctiva is anaesthetized with pantocaine or cocaine and the scraper is then drawn firmly over the conjunctiva, taking care not to cause bleeding. From each patient smears of cells were made on slides for examination to determine the presence of inclusions, and the remaining cells were washed off the spatula into physiological saline or into 5 per cent. peptone water and kept as near to 4° C. as possible for virus isolation. Unless otherwise stated, all material stored for virus isolation had penicillin 50 units and streptomycin 100 u/g. per ml. added to the suspending fluids to inhibit bacterial growth.

Infection of eggs or of tissue cultures was performed as soon as material was shown to contain inclusions. In Jordan and in London this might take from a few minutes up to some 18 hrs, with material that required overnight staining, to identify inclusions. The Gambian material was flown to London for virus isolation and this entailed a flight of 36 hrs; whenever possible the material was freeze-dried before dispatch and was packed in ice so that on arrival in England the temperature was from 5–7° C. Freeze-drying followed the method of Collier (1955). Before use the freeze-dried material was reconstituted with distilled water.

At first, smears for the identification of inclusions were fixed in methyl alcohol for from 2 to 5 min. and then stained in a mixture of 1 per cent. Giemsa and 0·5 per cent. May Grunwald stain in phosphate buffer at pH 6·8 for 16 to 18 hrs; after staining, the slides were rinsed rapidly in 95 per cent. alcohol and then in phosphate buffer and allowed to dry. The dry film was examined for trachoma inclusions.

It was found that the Giemsa staining method was too slow and laborious, and to speed up diagnosis the iodine staining method of Rice (1936) was used. By this method cells were allowed to dry on the slide and then treated with 2·5 per cent. iodine in 5 per cent. potassium iodide for 2 to 5 min., and then blotted dry. The dry film is covered with immersion oil and examined with a 2/3 objective when the inclusions stand out a mahogany brown against the background of pale yellow cells; confirmation of an inclusion can be made with an oil immersion objective when the typical granular nature of the inclusion becomes evident. (A more detailed account of the method is to be published.)

Methods of Attempted Isolation

Fertile chick embryos were inoculated by the following routes:

(*a*) 11 to 13-day-old embryos were inoculated on the chorio-allantoic membrane, using the false air sac technique.

(*b*) Amniotic inoculation was performed in 11 to 12-day embryos.

(*c*) Using 13-day embryos, injection was made by means of a tuberculin syringe directly into the brain or eye of the embryo, under direct observation. As the mortality of the method is high all deaths within 36 hrs were considered to be non-specific.

(*d*) Yolk-sac inoculation was used because of the morphological similarity of the inclusions of trachoma and viruses of the Psittacosis group. Embryos were incubated for from 3 to 9 days before infection. In younger embryos, of from 3 to 7 days' incubation, inoculations were made blindly into the yolk sac. With older embryos a false air sac was produced and inoculation made directly into the yolk sac.

Eggs were observed for 7 days and, if at the end of that time no lesions were found or death had occurred, the material was ground in saline and passed to a further batch of eggs. It was customary to make either two or three blind passages in batches of three or four embryos before a specimen was considered to be negative.

Adult mice of a "virus-free" albino strain were inoculated intracerebrally under ether anaesthesia (vol. 0·02 ml.). The mice were watched for 14 days, after which the brains were removed aseptically and a 10 per cent. brain suspension passed intracerebrally to more mice. Only two blind passages were made in mice.

As suckling mice are more susceptible to virus infections, groups of mice from a few hours up to 7 days old have been inoculated either by the subcutaneous route (using 0·2 ml.) or intracerebrally (using 0·01 ml.). Intracerebral inoculation entails some mortality and all deaths within 24 hours were considered to be traumatic.

In a further attempt to increase the susceptibility to the virus, suckling mice were given 2 mg. cortisone, which was found to be the maximum dose tolerated, at the time of inoculation. In all these experiments a paired litter of mice was given cortisone alone and the mortalities in the two groups were compared, as an occasional death sometimes occurred as a result of the cortisone alone.

Tissue Culture

(*a*) Epithelial cells removed by scrapings were taken up in Earle's saline; on arrival at the laboratory they were centrifuged at 1,000 r.p.m. for 1 to 2 min., until a small pellet of cells was obtained. The pellet was removed and placed on a sterile coverslip and then embedded in a clot of either human or chick plasma. The culture was set up in depression slides. Using this technique, cells appeared to remain unchanged for up to 7 days.

(*b*) HeLa cells were cultivated in a medium of the following composition:

Lactalbumin hydrolysate	5 ml. of 5 per cent. solution
Human serum	20 ml.
Eagle's amino-acid mixture in Earle's saline	75 ml.

The pH was adjusted to 7·4 with sodium bicarbonate containing phenol red, and antibiotics were added. The antibiotics were penicillin 100 units, streptomycin 100 u/g. and mycostatin 25 units, to each ml. medium; in later experiments Neomycin was added, as this inhibits the pleuropneumonia-like organisms that have been demonstrated by Collier (1957) in HeLa cell cultures.

In maintenance medium for HeLa cell cultures, human serum was replaced by horse serum and lactalbumin was omitted.

Material was inoculated into three tubes and cells were observed daily for cellular changes; one tube was sacrificed at weekly intervals and fixed and stained by

Giemsa stain to attempt to demonstrate inclusions. As it was felt that the antibiotics might inhibit viral growth, several cultures were set up in their absence, but difficulty with bacterial and fungal contamination was encountered.

In addition to simple addition of the inoculum to the culture of HeLa cells, a more intimate mingling of cells was attempted by culturing the conjunctival cells in a suspension of HeLa cells.

In a few instances serial blind passage of material was attempted in HeLa cells.

(*c*) A strain of Chang's conjunctival cells obtained from Dr. J. C. Snyder was cultured in the following medium:

Human serum	20 per cent.
Chick extract	5 per cent.
Earle's amino-acid saline	75 per cent.

The pH was adjusted to 7·4 and antibiotics added as for HeLa cells. It was found that these cells degenerated in the absence of human serum and hence it was necessary to add 5 per cent. human serum to the maintenance fluid.

The inoculation procedures were the same as for HeLa cells.

(*d*) In certain cases of trachoma, the tarsal plate and overlying conjunctiva are removed at operation and use was made of this material to attempt to grow the trachomatous epithelium. Two methods were used:

(*i*) The fragment removed at operation was received in Earle's saline, this was cut into pieces of about 2 mm. square and imbedded in a plasma clot in a test tube. After overnight incubation to harden the clot, growth fluid as used for conjunctival cells was added. Tubes were incubated without rolling and the fluids were changed every 48 hrs.

(*ii*) The method of Medawar and Billingham (1951) for the removal of fibrous tissues from skin grafts was used. Tarsal fibrous tissue was digested until it could be stripped from the overlying epithelial sheet, which was then cut into fragments and treated in the same way as the indigested fragments. Unfortunately, it proved impossible to carry digestion to a stage where only epithelium remained, as we found that no cellular multiplication occurred in such explants, trypsin apparently having damaged the epithelial cells.

(*e*) On two occasions, inclusion-rich material was inoculated into the conjunctiva of a human volunteer. The technique was to cocainize the eye and rub the inoculum into a previously scarified epithelium of the upper tarsal conjunctiva. The conjunctiva was examined for changes using the slit lamp, and scrapings of epithelium were examined for the development of inclusions.

Clinical Material

One of the problems of trachoma is the diverse clinical conditions to which the name trachoma is applied. Because of this we limited our investigations to cases which fulfilled the criteria laid down by the WHO Expert Committee on Trachoma (1955). This committee stated that two of the following four features must be present before the diagnosis can be made:

(1) Tarsal or limbal follicles
(2) Epithelial keratitis
(3) Pannus
(4) Typical scars

However, it appeared to us that, even using these clinical features, more than one condition might be included, and for our isolation studies we also demanded the presence of typical trachomatous inclusions in the epithelial cells.

Since the areas in which this work was performed are widely separated, it is well to point out some of the differences in the disease in these areas. The cases seen in London were all old cases, and where inclusions were found they were almost all recrudescences of an old infection, the longest period between primary infection and relapse being 20 years, although it is possible this may have been a re-infection.

In Jordan, estimates of the trachoma incidence have been as high as 85 per cent. (Shusha, 1956), but by our standards of diagnosis we found the incidence much less than we had expected; the difference in estimates may be attributable partly to early treatment, but probably a more important factor is the common practice of designating all cases of chronic conjunctivitis as trachoma. Typical clinical trachoma was found in only 2 per cent. of hospital outpatients, and in only 20 per cent. of these was it possible to demonstrate inclusions in scrapings. Another impression that we gained from Jordan was that any form of antibiotic treatment greatly reduced the incidence of inclusions; thus active trachoma was much less common in refugee camps, where penicillin and streptomycin ointment was available free, than in villages where no free medical aid was available.

The clinical picture in Gambia showed some differences from those in Jordan, and the most striking of these was the relative absence of pannus from many of the cases which showed gross lid involvement. In the areas examined it was found that about 45 per cent. of the population had the WHO criteria of trachoma, and in about 20 to 25 per cent. of these it was possible to find cytoplasmic inclusions.

Results

The results of attempts at isolation are considered under each of the routes used:

(1) Chick Embryo

(*a*) *Chorio-allantoic Membrane.*—Six inclusion-positive scrapings were set up, each in batches of four eggs. An occasional lesion was seen in the primary culture but proved to be non-transmissible, and smears from the lesion stained by Giemsa stain failed to show the presence of inclusions.

(*b*) *Amniotic Inoculation.*—Four scrapings from cases showing inclusions were inoculated by this route; deaths were not more numerous than in control groups of eggs, and examination of embryos at 5 to 7 days failed to reveal any specific lesion.

(*c*) *Allantoic.*—Scrapings from three cases of trachoma were put into the allantoic cavity of batches of six embryos. No deaths occurred and tests for haemagglutination were negative, while stained smears did not show the presence of inclusions.

(*d*) *Visceral.*—The results of attempts to demonstrate virus by inoculation into the eye or brain of an embryo were negative.

(*e*) *Yolk Sac.*—In all, twenty positive scrapings were inoculated by this route in batches of four or six embryos. No greater death rate occurred than in saline controls, and yolk sac smears stained by Giemsa–May Grunwald stain and by Casteneda's stain did not show the presence of viral inclusions.

(2) **Mice.**—In neither adult nor in suckling mice could evidence of infection be found. No latent virus was unmasked by passage into a second group of animals.

In three of four groups of suckling mice in which cortisone was given with the inoculum, the mice receiving the trachomatous material died, while those receiving cortisone alone did not. Unfortunately, the carcases were usually eaten by the mother, but those in one litter were killed when moribund, and in these no lesions which could be attributed to trachoma were found; the *post-mortem* appearances being similar to those seen by Antopol (1950). The carcases of these mice were ground up in saline, and penicillin and streptomycin added, so that 10 per cent. emulsion of the mouse was made; inoculation of this suspension into a further batch of cortisone-treated mice gave a mortality similar to that in the control group receiving cortisone alone. It seems, therefore, that the initial deaths were due to some toxic factor acting synergistically with the cortisone rather than to any viral multiplication.

(3) Tissue Culture

(*a*) *Plasma Clot.*—Material from 10 cases with inclusions and 100 without inclusions was kept under observation by this method. In no instance was cellular multiplication observed, and neither was it possible to demonstrate the development of fresh inclusions.

In a few cases, preparations were also made on human serum agar, and watched by phase-contrast microscopy. No cellular change was seen that could be recognized as inclusion formation, but, owing to the lack of a hot box ciné-micrography apparatus, it was not possible to watch a single inclusion-containing cell over a period of time.

(*b*) *HeLa Cells.*—Material inoculated was all inclusion-containing, and with the exception of six batches from Gambia had all been inoculated with antibiotics:

25 batches inoculated from trachoma cases seen in Jerusalem
70 batches in which material was received freeze-dried from Gambia
40 batches in which material was received in Earle's saline from Gambia.

In three instances in Jerusalem, cytopathic degeneration suggestive of an adenovirus was seen, but owing to the interruption of work at the time of the Suez crisis the strains were lost and identification was not possible.

With the Gambian material it was striking that no cytopathogenic agents were isolated, as it was expected that both adenoviruses and herpes simplex which cause these changes would be encountered.

(*c*) *Conjunctival Cells.*—In view of the report of Murray and others (1957), in which isolation had been attempted from 600 cases of trachoma, only six positive scrapings were inoculated in these cells. Our findings like those of

Murray were negative. In our hands these conjunctival cells look most unlike the conjunctival cells in which trachomatous inclusions are normally found.

(*d*) *Tarsal Plate Cultures.*—Our experience with scrapings suggested that the superficial epithelial cells were incapable of multiplication, and also, judging by pH change in tissue culture media, had very little metabolism. Hence it was decided to try to culture the deeper layers of cells from the infected material found in tarsal plates removed at operation. It was hoped that, if epithelium grew out from such material, the fresh cells would become infected, and this would be made manifest either by cellular necrosis as was found by Rowe, Huebner, Hartley, Ward, and Parrott (1953) with outgrowths of tonsillar epithelium and adenoviruses, or by the appearance of inclusions in the cells. Unfortunately, most of the cellular proliferations from the explants were of fibroblasts, and in only six fragments did epithelium proliferate. In these six, the epithelial outgrowth occurred between the fifth and tenth day after the culture was set up and appeared as a fringe around the implant. Four of the explants showing epithelium were set up by the trypsin technique, and two were fragments directly implanted in plasma clots.

In all cases in which epithelium appeared to proliferate, growth was maintained for about 5 to 10 more days when, quite abruptly, the new cells were shed off the parent explant and underwent necrosis; no specific cellular degeneration appeared to precede the necrosis, although frequently the plasma clot in which the epithelium was growing commenced to undergo digestion at this time. Repair of the plasma clot or re-implantation of the fragment usually led to a secondary multiplication of fibroblasts.

It is essential to differentiate between the true outgrowth of epithelium and the large numbers of free rounded epithelial cells which appear round the explant during the first 72 hours. These latter cells are derived from the superficial layers of the epithelium, and like those removed by scraping do not appear to be able to multiply.

Two types of fibroblastic cells grow from the explants which do not form epithelium. The first to appear are small elongated cells which radiate from the explant like spines of a porcupine. Later, both within the area covered by these cells and in that peripheral to them, a larger fibroblastic cell appears, which shows a more random arrangement and often possesses three processes as against two in the smaller cells. Both these types of cells were kept alive in culture for up to 10 weeks, but during that time no evidence of damage (due to virus infection) appeared.

Attempts to obtain cultures of fibroblasts and of epithelium from conjunctiva that could be grown in serial culture were unsuccessful.

In order to demonstrate the absence of inclusions, tube cultures were washed in saline and then fixed in equal parts of absolute alcohol and ether, after which they were stained by the Giemsa–May Grunwald stain. Cultures

stained in this manner could be examined wet, or dried and examined after the tube had been filled with liquid paraffin; although tube preparations can only be examined by low-power objectives the typical trachomatous inclusion can readily be recognized at this magnification.

(*e*) *Complement-Fixation with Tissue Culture Fluids.*—Serum was obtained from a number of patients with clinical Stage II or III trachoma; these sera were pooled and inactivated at 56° C., and used to test tissue culture fluids for the presence of complement-fixing antigens.

In many instances tissue culture fluids proved highly anticomplementary, and it became necessary to dilute the antigen 1:5 before it could be used.

The following fluids were tested:

20 from HeLa cell cultures
6 from tarsal plate culture in which epithelium was necrosing
2 from conjunctival cell cultures.

In none was it possible to demonstrate significant evidence of complement-fixing antigens, although with one fluid from a tarsal plate there was a suggestion of partial fixation two tubes higher than the control; in view of the known anticomplementary nature of the tissue culture fluid, this was not considered to be significant.

(3) **Human Inoculation.**—On two occasions a volunteer was inoculated with material from patients containing many inclusions, which had been received freeze-dried. The subject was repeatedly examined for evidence of infection, but the only change noted was some discharge for 48 hours after inoculation which could be accounted for by the trauma of the procedure. There was no development of follicles and no corneal involvement, while scrapings failed to show the presence of trachoma inclusions.

Discussion

Despite our failure to isolate the trachoma virus, many points of interest arise from our results. The first is the confirmation of the many negative findings recorded in the literature, and the inability to obtain results similar to those of workers who have claimed to isolate the trachoma virus. In general, it seems that neither chick embryos nor mice are capable of supporting the multiplication of the virus, although it is possible that it may remain viable in the tissues without multiplying as has been suggested by the work of Stewart and Badir (1950), who maintained the virus by alternate passage in monkeys and eggs.

The claims to have isolated the virus by egg or mouse inoculation could be explained by this persistence of non-multiplying virus, or by the presence of another virus in the inoculum; this could well be the case with an adeno-virus which one of us has found to persist for up to three passages in the

chick embryo. It is well to remember that to prove a virus to be the cause of a disease it is necessary to fulfil certain criteria which are as demanding as "Koch's postulates". These have been summarized by Jawetz, Thygeson, Hanna, Nicholas, and Kimura (1957), in their discussion on the aetiology of epidemic kerato-conjunctivitis, and so far no agent which will satisfy the requirements has been isolated from a case of trachoma; for example, none of the agents isolated has been made available to other laboratories so that the original findings can be confirmed by other workers.

With the material flown to London from Gambia, there is the criticism that the virus might not be viable after a 36-hr plane journey, and in favour of this is the inability to produce infection in a human volunteer. On the other hand it was possible to cultivate both epithelial and fibroblastic cells from the material, and it is difficult to imagine that the virus within a cell is more susceptible to trauma than the cell which it parasitizes. Work is still necessary to clarify this point, as the human experiments could have failed through an inadequate technique of infection or a natural resistance to infection of the individual; conversely, it could be argued that intracellular virus was non-infective and that extracellular particles were killed in transit. In favour of this latter point is the original observation of Halberstaedter and Prowazek (1907), that infection of apes was more readily accomplished with material from trachoma patients with discharging eyes than from those with relatively clean eyes.

These points emphasize one of the fundamental difficulties in the investigation of trachoma, namely, the lack of any suitable means of identification of the presence of the virus. We have relied largely on cytopathology and the development of inclusions, but it is quite possible that neither of these may occur, as there are frequently wide differences between the behaviour of a virus in its natural host and in tissue culture. Thus varicella fails to produce free virus in tissue culture, and measles virus produces giant cells only when bovine amniotic fluid or glutamine are added to the culture media. The most satisfactory way of demonstrating virus in tissue culture would be the use of human volunteers, who would not be exposed to a great risk as the early stages of the disease can be so effectively treated by tetracycline antibiotics.

Considering the problem of trachoma in Jordan and in Gambia, it is apparent that bacterial infections are a much greater problem in Jordan than in Gambia. Thus, with the greater incidence of active trachoma in Gambia, the late complications such as trichiasis should be much more numerous, but in fact this is not the case. In Jordan there appeared to be also more viral infections other than trachoma than were found in Gambia. (One of us, M.J.G., however, did see a number of cases of clinical herpes simplex infections in the latter territory.) It seems to us that these differences are rather a reflection of the climatic and social conditions than any intrinsic modification of the disease. Thus the lesser bacterial infection may well be

due to the static agricultural life of the Gambians, as opposed to the nomadic life of the Arabs. Furthermore, the strict division of the Gambian village into compounds may also be important in this respect, as it was not unusual to find two compounds in the same village with widely differing incidences of infection; the lower rate of infection being found nearly always in the compound with the higher social status. Alimuddin (1958) has drawn attention to the much greater incidence of pannus in the dry areas of Pakistan as compared with the wetter areas. Our experience would agree with his findings, in that pannus is not a feature of trachoma in Gambia, where the rainfall is much higher than in Jordan. Another interesting observation is that the incidence of trachoma dropped as soon as piped water supplies became available. This applied in both Gambia and Jordan, and it was only while writing this paper that we became aware that Victoria (1947) had made a similar observation in Argentina.

The failure to isolate trachoma from operative material was a disappointment, as it was hoped that the necrosis of epithelial outgrowths might be due to viral action; unfortunately it proved impossible to demonstrate the presence of virus by staining, by complement-fixation tests, or by the passage of material to other cell lines. Although, in the absence of attempts to infect humans, this does not preclude the presence of a virus, it does suggest that it was unlikely that any appreciable quantity of virus was present.

Our results show a complete inability to isolate the virus, and it may be of interest to others attempting isolations to draw attention to some of the possible reasons for this failure:

(*1*) *Failure to Survive Transportation.*—Snyder (1956) found difficulty in transporting virus and it may be that in all our experiments there was too great a delay between the taking of scrapings and the inoculation into animals or tissue cultures —particularly with the material from Gambia, but this point can be settled only by investigation in volunteers. In this connexion it should be noted that Julianelle and Harrison (1938) found that the virus would remain viable for from 1 to 7 days in saline kept at 4° C., whereas at room temperature (20–24° C.) the virus remained active only for from a few hours up to 1 day. It seems unlikely therefore, that time alone was sufficient to inactivate our inocula.

(*2*) *Antibiotics.*—Both Bietti (1954) and one of us (M.J.G.) observed that the systemic administration of penicillin may bring about a clinical cure of trachoma, and it is generally acknowledged that the tetracycline antibiotics are effective against the disease; it seems therefore that all attempts at isolation may have to be made in the absence of antibiotics. This greatly increases the difficulties of isolation in tissue culture, and it might be rewarding to make careful trials of the effects of other antibiotics on patients with trachoma inclusions to discover a suitable combination of antibiotics which would suppress bacterial growth but not the growth of the virus, and so be suitable for use in tissue culture.

Julianelle and Smith (1942) found that sulphonamides did not inactivate the virus *in vitro*, and it is probable that other antibiotics might not inactivate the virus

except when it was multiplying intracellularly. If this were so, it might be possible to sterilize with antibiotics the conjunctival secretions collected, and then to remove the cells and virus from the antibiotics by centrifugalization so that no inhibitory material would be carried over into the tissue culture.

(*3*) *The Virus may not be Cytopathogenic.*—It is possible that the virus is not cytopathogenic or inclusion-producing in tissue culture, or that it is incapable of entry into the cells of the culture. In this connexion it might be worth investigating the action of lysozyme, which is normally present in high concentration in the conjunctival sac, and might facilitate the entry of virus into the conjunctival cells. Alternately, the cells may fail to release infective virus, as has been found with varicella virus in tissue culture.

(*4*) *Role of Inclusions.*—Although there is little doubt in our minds of the association of cytoplasmic inclusions with trachoma, there is a possibility that they may not represent the infective agent. In this connexion it should be remembered that inclusions are found in superficial cells that are relatively inert and, on the whole, normal in appearance, whereas the main pathological changes of the disease occur in the subepithelial layers in which follicle formation and intense cellular infiltration occur. To explain this it was suggested that a viral toxin might be formed. Toxins have been described in certain viral infections such as lymphogranuloma inguinale (Rake and Jones, 1943), but even in these cases the toxin could not readily be separated from the virus particle, and it is usual to suppose that the toxic reactions to a virus disease are due to viral growth damaging cells rather than to any hypothetical toxin. It seemed to us more possible that the inclusions represented an "incomplete" viral form, produced as through the limited metabolism of these superficial cells preventing complete development of the virus. If this were the case, it would be essential to obtain material from the deeper layers of the conjunctiva for virus isolation. It might imply also that cells which would enable the virus to multiply in tissue culture would not show viral inclusions.

(*5*) *Cell Type.*—Throughout the previous discussion it has been assumed that the cells used can support the multiplication of trachoma virus. Quite possibly this may not be the case, as viruses do not always multiply in the tissue apparently most closely resembling that from which they are isolated; thus cytomegalic inclusion virus grows in fibroblasts, and certain ECHO viruses from human sources will multiply in monkey kidney but not in human cells. Hence it may be necessary to obtain completely fresh cell lines, and this would seem to be one of the most fruitful approaches to the problem of isolating the trachoma virus.

Summary

It has proved impossible to isolate trachoma virus in HeLa cells, conjunctival cells, mice or chick embryos. Some of the possible reasons for failure are discussed.

We are indebted to Drs. L. Collier, B. Andrewes, and J. Snyder who have supplied us with cultures of HeLa and other cells and to Mr. I. Barnett and Miss A. Fischer for their technical help.

Our gratitude is due to W/Cdr. J. G. Price and Dr. A. Boase, Wardens of St. John's Ophthalmic Hospital, Jerusalem, to Dr. C. Ross Smith and the medical staff of U.N.R.R.A., Jerusalem, and to Dr. I. MacGregor and the staff of the Medical Research Council, Gambia.

Finally we are grateful to Sir Stewart Duke-Elder without whose enthusiasm this work would not have been possible.

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Brit. J. Ophthal. (1958) **42**, 473.

STAINING OF THE INCLUSION BODIES OF TRACHOMA AND INCLUSION CONJUNCTIVITIS*

BY

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THE first description of the inclusion bodies of trachoma was made by Halberstaedter and von Prowazek following the examination of Giemsa-stained scrapings from the conjunctivae of baboons which had been inoculated with secretions from trachomatous patients (*cf.* Julianelle, 1938). Since then Giemsa and its modifications have held prime place as a means of demonstrating these structures and this position has been upheld by the recommendation of its use in the Second Report of the WHO Expert Committee on Trachoma (1955): "for trachoma studies—for the examination of smears and scrapings from the conjunctiva".

Although Giemsa may well constitute a standard reference of staining, certain problems arise from its use: first, the prolonged staining time and the need for solutions of controlled pH; secondly, the superficial similarity between the Giemsa-stained HP inclusion and a number of other intracellular appearances (e.g. granular cytoplasm, nuclear prolapse), which, even in experienced hands, militates against the speedy examination of smears; thirdly, the difficulty of detecting HP inclusions in thick clumps of tissue; finally, the difficulty in distinguishing the full details of inclusion morphology when this overlies a deeply-stained cell nucleus.

These factors have led to a search for suitable alternative stains. Among these are Wright's modification of Giemsa, Castaneda's stain, Lindner's contrast stain, Macchiavello's stain, and Poleff's stain. Of these most have proved of no greater value than Giemsa, while others, such as Poleff's methylene blue, have been found unreliable (Thygeson, 1955), owing to the ease with which various intracytoplasmic structures may simulate HP inclusions.

Despite the recommendation in the First Report of the WHO Expert Committee on Trachoma (1952) ". . . the development of rapid methods for the detention of inclusion bodies: Thick scrapings. Iodine stain . . .", little further work appears to have been done, except that of Dark (1955), confirming the earlier studies of Rice (1936) and Thygeson (1938), on the staining by iodine of the glycogen-containing matrix of the HP inclusion.

During the course of a recent project on the aetiology of trachoma this problem of speedy and accurate detection assumed prominence as it was

* Received for publication February 28, 1958.

necessary to demonstrate the presence of HP inclusions in conjunctival scrapings, before their inoculation into tissue culture. In the case of negative material, full microscopy of Giemsa-stained smears from both eyes was found to require up to one hour per slide. The possibility of inclusion-positive cases being discarded owing to inclusions being missed in thick clumps of tissue was also present. Following unsatisfactory trials of the Castaneda and Macchiavello stains, it was decided to experiment with the iodine-staining method of Rice (1936).

Methods

In our hands the technique reported by Rice (1936) and Thygeson (1938) proved unsatisfactory. The staining was faint and liable to fade. Acidification of the iodine solution, advocated by Rice, gave no improvement.

Some of the difficulty lay in the strength of the Lugol's solution—in the earlier reports the strength is not stated. Gatenby (1956) has enumerated the various strengths of solution which have been described under this title, ranging from 0·33 to 5 per cent. iodine in aqueous, or even alcoholic, solutions of potassium iodide from 2 to 10 per cent. It would seem probable that this confusion has resulted in other workers using insufficient concentrations with correspondingly disppointing differentiation of HP inclusions. Initially we had been using bacteriological Lugol (0·33 per cent. I_2 in 2 per cent. KI aqueous solution) but transfer to clinical Lugol (5 per cent. I_2 in 10 per cent. KI aq.) yielded much more favourable results.

The use of unfixed material was found to give greatly improved results in terms of definition, density and duration of staining with no tendency for the scraping to become detached from the slide, presumably owing to the fixative action of the iodine. Alcohol fixation was found to have a particularly deleterious effect and the presence of small amounts, even as vapour, after staining, caused rapid decolourization.

After further investigation a final technique was evolved, and this has been used on several hundreds of slides of scrapings from trachomatous patients in Jordan and Gambia. The appearances reported have also been found to be valid in three cases of inclusion conjunctivitis seen in the U.K.

First, scrapings are smeared on clean dry slides and allowed to dry in the air for at least 5 minutes; they are covered with 5 per cent. iodine in 10 per cent. KI aqueous Lugol's solution which is left *in situ* for some minutes, care being necessary to avoid drying and crystallization of the stain on the slide. A period of 2 minutes is normally sufficient with fresh material, but longer times may be required with dry specimens. If overstaining occurs, the solution may be diluted with water, 1:1. The excess iodine is then shaken off and the slide pressure-dried between two pieces of filter paper (filter paper is preferable to blotting paper as the improved absorption minimizes crystallization). The smear is then covered with immersion oil, this acting as a clearing agent and delaying fading from sublimation of the iodine especially with fresh material. The slide may then be examined with either the 2/3″ or the 1/12″ oil-immersion objective, no cover-slip being required.

On completion of microscopy, the oil is removed by several washings with toluol and the slide may be kept for some months for repeat staining. Alternatively,

decolourization before staining with Giemsa or Wright's stains is carried out by one of the following methods:

(*i*) Wash thoroughly in absolute or 95 per cent. methyl alcohol—which also acts as a pre-Giemsa fixative. The longer the slide is left stained with iodine the longer washing should be prolonged, up to several hours.

(*ii*) Wash in 0·5 per cent. solution of sodium hyposulphite for 10–15 minutes followed by prolonged washing in tap and distilled water. The first method is preferable, in that the addition of further chemicals which may crystallize is avoided, while at the same time the slide is fixed for further staining. Slides so decolourized may be re-stained with iodine several times, but after a period of weeks or months the staining becomes less certain, possibly owing to dessication. If decolourization is effectively carried out and not unduly delayed, no harmful effects on the subsequent use of other stains have been observed. Prolonged delay, particularly in fixation with alcohol, may result in unsatisfactory colour toning with the polychromatic stains.

Examination

The scraping as a whole is stained a fairly uniform golden-yellow against which, especially by condenser adjustments, the various types of cell—epithelial, mononuclear, polymorphonuclear cells, and red blood corpuscles—may be distinguished through the 1/12″ objective by their size and nuclear outline.

HP inclusions, when present, stain a deep orange-brown and stand out clearly against the background, so that with a little experience they may readily be detected with the 2/3″ objective, recourse being had to a higher power for confirmation. In detail they are seen as dotlike masses (although it is the matrix rather than the virus particles which is believed to stain with iodine) within the cytoplasm of epithelial cells, paranuclear but also often forming a network which encloses the nucleus. Rarely, an inclusion from a ruptured cell may be observed as an area of scattered staining dots in the vicinity of the affected cell.

In single and isolated cells, the structure of the inclusions may be studied in detail, and even when situated in a layer several cells thick the distinctive colouring and granularity are readily detected, although the finer detail may be obscure.

Intracellular melanin is visible as dark green-brown dots, coarser than the HP inclusions and usually of circum-nuclear distribution. Brown-staining clumps and crescents, non-granular in character and of a browner tint than the HP inclusions, may be present in the cytoplasm of polymorphonuclear cells. Crenated red blood corpuscles present a similar appearance. Occasionally, in the presence of severe bacterial and fungal infection, large clumps of amorphous staining material may be seen in the cytoplasm of large epithelioid cells which may be tissue macrophages; these clumps probably represent phagocytosis of degenerate leucocytic material. All these appearances may superficially resemble HP inclusions under the 2/3″ objective,

but the use of the 1/12″ oil-immersion lens will disclose their true nature. Such identification can be made after considerably less experience than is required to identify HP inclusions in a Giemsa-stained preparation. Where serious doubt as to the nature of a staining object arises, the field should be located by vernier or "microviseur" readings and examined after restaining with Giemsa. However, not infrequently, the object cannot then be clearly distinguished against the deeply-stained background of a thick layer of tissue.

Discussion

Some of the particular advantages of the method have been discussed by Thygeson (1938) and it is felt that the modifications described accentuate these. They may be set out as follows:

(1) Speed, ease, and simplicity in staining and examination. With a little experience scrapings may be taken and stained, and an opinion on the presence or absence of inclusions given in 15 minutes. In consequence, the method would appear to have special advantages for field work.

(2) The ability to identify inclusions in thick smears, which would be missed by other methods. In our series of several hundred scrapings examined, between 25 and 30 per cent. of all inclusions seen would not have been visible in Giemsa preparations, and from 5 to 10 per cent. of all slides would have been labelled inclusion-negative.

(3) The morphology of the inclusions may be seen in greater detail, especially in regard to the relationship with other intracellular structures, *e.g.* nucleus, Golgi apparatus. Studies on this aspect of inclusion morphology might elucidate the peculiarly specific "helmet" shape which HP inclusions in general tend to assume.

The main disadvantage of the technique lies in the possibility that not all HP inclusions possess a glycogen-containing matrix, on the presence of which staining depends. It has been suggested that this may be absent in the extracellular phase and in the earliest stages of intracellular formation.

In practice it is doubtful whether this objection is valid. Smaller inclusions than may reliably be identified with Giemsa are readily seen, while the identification of extracellular virus particles by either method, except when in the vicinity of a ruptured inclusion containing cells, is a matter for uncertainty.

The question remains whether the method permits the visualization of initial body inclusions. Here the position is uncertain and will depend on the view taken of what is an initial body inclusion when Giemsa's stain is used.

Whether inclusions produced experimentally in the non-human conjunctiva, tissue culture, or yolk sac can be demonstrated with this technique is conjectural, but this would seem an appropriate subject for further study.

Conclusions

The method described presents a simple way of staining and identifying the inclusion bodies of trachoma and inclusion conjunctivitis which should prove of especial value in field studies, particularly in connection with attempts at the culture of these viruses, so long as the only generally accepted evidence of infectivity is the presence of the specific HP inclusions. Similar considerations apply in connection with the conduct of therapeutic trials.

Summary

A modification of Rice's method (Rice, 1936) of staining the inclusion bodies of trachoma and inclusion conjunctivitis is described.

The potential advantages of this method over the Giemsa type stains recommended by the WHO Expert Committee on Trachoma, particularly for certain aspects of trachoma research, are discussed.

The facilities and assistance given by the Director and Staff of the Medical Research Council Laboratories, Fajara, Gambia, are gratefully acknowledged. Similar facilities were afforded by the Order of St. John of Jerusalem, in Jordan. Our thanks are due to Sir Stewart Duke-Elder, Honorary Director of the Group, for helpful advice and criticism.

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Brit. J. Ophthal. (1958) 42, 478.

TRACHOMA INCLUSIONS AND PENICILLIN*

BY

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AFTER the release of penicillin for general medical use after the second world war, immediate interest was awakened in its possibilities in the therapy of trachoma. The subject was extensively investigated but results tended to be equivocal and attention became diverted to the newer antibiotics as these became available. In consequence, no definite assessment of the potentialities of penicillin in this disease appears to have been made.

However, developments in the technique of virus isolation by tissue culture methods have raised the question of the action of pencillin on trachoma to a new importance, in view of its use, with or without other antibiotics, to prevent the contamination of cultures. Recently Tang, Chang, Huang, and Wang (1957), reporting the culture in hen eggs of a virus, probably that of trachoma, have reported the inhibition of viral growth by such use.

The following observations of the disappearance of HP inclusions from the conjunctival scrapings of a small series of cases of trachoma, previously inclusion-positive on repeated examination, after treatment with penicillin, may therefore be of interest.

Our attention was drawn to this in the course of work on the aetiology of trachoma in Jordan, when a tendency for inclusion-positive cases to become inclusion-negative, after minimal local or general therapy with sulphonamides or antibiotics, was observed. Having regard to the likelihood of such disappearance occurring in the later stages of the disease, even in the absence of therapy, little weight was attached to this.

After this work was transferred to Gambia, the observations reported below in Case 1 were made, and in view of the very definite changes noted it was decided to attempt their reproduction in a small series.

Methods

All patients were African of Mandingo or Jola extraction. With the exception of Case 1 they had been followed-up by repeated conjunctival scrapings for some months before the present observations were made. Inclusions were found to be present at every examination.

Patients were admitted to hospital and conjunctival scrapings were taken from

*Received for publication February 28, 1957.

the upper tarsae every second day, using alloy scrapers without the previous administration of local anaesthetic—in our experience this procedure is well tolerated. Clinical details were confirmed with the slit lamp (except in Case 4). In most cases bacteriological cultures were taken before each scraping and organisms identified by cultural characteristics and microscopy.

After admission, pencillin was administered to Cases 2 to 5 in the form of aqueous suspension of procaine penicillin (Avloprocil), one injection of 900,000 units daily for 5 days. No other local or general therapy was given.

Inclusions were identified by the iodine-staining method of Rice (1936) as modified by Gilkes, Smith, and Sowa (1958). Where necessary such identifications were confirmed by re-staining with Giemsa.

Case Reports

Case 1, a male infant aged 4 months, had clinically early Trachoma Stage I. There were many small follicles and papillae, but, as is commonly found in Gambian trachoma, no evidence of any corneal involvement.

27.5.57: He was admitted to hospital for repeated conjunctival scrapings to observe the alleged developmental cycle of the inclusion body. Culture was sterile. Scrapings showed inclusions in vast numbers (as many as nineteen in one 1/12" objective field).

30.5.57: Scrapings showed inclusions greatly reduced in number. The majority of those seen were increased in size and vacuolated in appearance. Epithelial cells were noted as containing iodine-staining fragments suggestive of disrupted inclusions.

3.6.57: Clinical signs fewer and conjunctival congestion reduced. Scrapings showed no inclusions, but a few leucocytes containing stained granules.

5.6.57: Lids clinically normal. Scrapings showed no inclusions.

5.7.57: Lids clinically normal. Culture showed few *Corynebacterium* and *Staph. albus.* Scrapings were normal, with epithelial cells only.

On investigating this sudden disappearance of inclusions, it was discovered that from 29.5.57 to 31.5.57 the child had received three daily intra-muscular injections of procaine penicillin 900,000 units for treatment of a concurrent scalp infection. No other local or systemic treatment had been given.

Case 2, a boy aged 4 years, had Trachoma Stage I. There were scanty follicles and papillae, but no pannus. Repeated scrapings from 27.2.57 showed inclusions constantly present, associated with a trachomatous type of cytology.

6.5.57: He was admitted to hospital. Culture showed a scanty growth of Koch-Weeks bacilli and *Corynebacterium.* Scrapings showed inclusions ++. Penicillin treatment was commenced.

9.6.57: Scrapings showed fewer inclusions, which were large, vacuolated, and degenerate in appearance.

11.6.57: Culture was sterile. Scrapings showed no inclusions, but a few inflammatory cells seen.

13.6.57: Culture was sterile. Scrapings showed no inclusions. The patient almost clinically normal.

5.7.57: Culture was sterile. Scrapings showed normal epithelial cells only. Patient clinically normal.

Case 3 a girl aged 12 years, had Trachoma Stage II with many follicles, few papillae, and no pannus. Repeated scrapings from 6.3.57 had shown inclusions associated with a trachomatous type of cytology.

6.5.57: She was admitted to hospital. Culture showed a heavy growth of *Staph. pyogenes* and a scanty growth of *Corynebacterium*. Scraping showed many inclusions. Penicillin treatment was commenced.

8.6.57: Scrapings showed few inclusions, which were large, vacuolated and degenerate.

12.6.57: Culture was sterile. Scrapings showed no inclusions, and nearly normal epithelial cells containing iodine-staining granules.

14.6.57: Culture was sterile. Scrapings showed no inclusions. The patient was clinically nearly normal.

5.7.57: Culture showed a heavy growth of pneumococci and a fair growth of *Staph. albus*. Scrapings showed normal epithelial cells only. Patient clinically normal.

Case 4, a girl aged 5 yrs, had Trachoma Stage II, with few follicles, many papillae, and 1·5 mm. pannus tenuis. Scrapings from 19.12.56 constantly showed inclusions to be present.

18.6.57: She was admitted to hospital. Culture showed a fair growth of *Corynebacterium*. Scrapings showed many inclusions. Penicillin treatment was commenced.

20.6.57: Culture showed a scanty growth of *Staph. pyogenes*. Scrapings showed no inclusions, but a few inflammatory cells.

22.6.57: Culture showed a heavy growth of Koch-Weeks bacilli. Scrapings showed no inclusions. Clinically the patient was much improved.

24.6.57: Culture showed a scanty growth of *Staph. albus*. Scrapings showed no inclusions.

5.7.57: Culture showed *Staph. albus*. Scrapings showed normal epithelial cells only. Patient clinically normal.

Case 5, a boy aged 12 yrs, had Trachoma Stage II, of the proliferative type, with gross papillae, a few follicles, 1·5 mm. pannus, and Herbert's pits. Scrapings from 27.2.57 showed inclusions constantly present, sometimes in vast numbers (up to thirteen in one 1/12″ objective field).

6.6.57: He was admitted to hospital. Culture showed a scanty growth of *Corynebacterium* and *Staph. albus*. Scrapings showed many inclusions present. Penicillin treatment was commenced.

8.6.57: Scrapings showed fewer inclusions, which were large, vacuolated, and degenerate in appearance.

11.6.57: Culture showed a scanty growth of *Corynebacterium*. Scrapings showed no inclusions, but some epithelial cells with iodine-staining granules. Clinically the patient was much improved.

13.6.57: Culture was sterile. Scrapings showed no inclusions, but mainly normal epithelial cells.

5.7.57: Culture showed a scanty growth of *Corynebacterium*. Scrapings showed no inclusions, but some inflammatory cells were present. Clinically the patient was much improved. There were some residual papillae.

In conjunction with this series, other cases of Trachoma Stage II and III, in which inclusions had been consistently present for 3 or more months, were treated as outpatients with local applications of penicillin and streptomycin ointment (Mystrepton. Penicillin 2,000 units/g.: Dihydrostreptomycin sulphate 10,000 units/g.) twice daily. In three of these, all siblings, vacuolated degenerative changes were observed in the inclusions, which later disappeared and had not recurred up to one month later. In the remaining three cases, after transient changes, the inclusions persisted. The cultures became sterile in the former three but remained positive in the latter.

Discussion

It has not been possible to prolong the follow-up of these cases or to enlarge the series to a more satisfactory size, but substantially our findings confirm those of Bietti (1948, 1949), who reported degenerative changes in the inclusion bodies of trachoma after the local application of penicillin, using Giemsa stain.

The changes were specific and constant in character, occurring on the second or third day of treatment, associated with a definite reduction in the number of the inclusions, and preceding their total disappearance. Such changes have not been observed in the absence of therapy.

The present series, however, differs from that of Bietti in two respects:

(1) The use of the iodine-staining method, which showed the morphological changes in the inclusions more clearly than when Giemsa or Wright's stains are used. With these latter stains the later degenerative changes become increasingly difficult to differentiate from other appearances not connected with the inclusions, which may be present in the cytoplasm of the epithelial cells.

(2) The use of a very large short-term systemic dosage unaccompanied by local therapy.

Bietti (1951) stated that in trachoma local application is the most efficacious form of therapy, and this view was supported and quoted by Nataf (1952). More recently, Bietti (1955) has acknowledged the possibility that a considerably larger systemic dosage might be effective and favourable results with a dosage smaller than ours have been reported by several authors, including Siggia and Mamola (1950), Scuderi and Bellomio (1950), and Zeppa (1951). Bietti and Pannarale (1955) have also reported encouraging results, with the occurrence of degenerative inclusion changes, following repository injections of N-N diethylenediaminedibenzylpenicillin G (DEBP), again with a smaller effective daily dose than in our series.

In view of the small size of this series, no attempt is made to present the reported regime of dosage as curative of trachoma. None the less the definite clinical response and the changes in the inclusions indicate that further investigation of the therapeutic possibilities of heavy systemic dosage merits consideration.

It is rather our purpose to suggest that the use of penicillin in connexion with any procedures directed to the cultivation of the trachoma virus should be avoided. Degenerative changes in the inclusion bodies have been reported following the clinical use of the newer antibiotics, Terramycin, chloramphenicol, and Aureomycin, and similar considerations may apply in connexion with these substances.

Summary

The degenerative changes and the disappearance of inclusion bodies in a small series of longstanding inclusion-positive cases of trachoma after short courses of massive systemic daily dosage with penicillin are described.

The significance of these findings in relation to the use of antibiotics in the attempted tissue culture of the trachoma virus is discussed.

The facilities and assistance afforded by the Director and Staff of the Medical Research Council Laboratories, Fajara, Gambia, and especially the help and co-operation of Sisters Baird and Ball, are gratefully acknowledged. The conscientious examination of many slides by our assistant Miss A. Fischer greatly facilitated the investigation. The work was carried out under the Honorary Directorship of Sir Stewart Duke-Elder whose helpful criticism is gratefully acknowledged.

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Brit. J. Ophthal. (1958) **42**, 483.

MODE OF ACTION OF TARSORRHAPHY IN NEUROPARALYTIC KERATITIS*

BY

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LITTLE is known about the mode of action of tarsorrhaphy in neuroparalytic keratitis, though this simple operation is followed by a dramatic improvement, sometimes in a matter of hours. Neuroparalytic keratitis may result from diverse causes, the common factor being a lesion of the trigeminal ganglion.

The theory of the causation of neuroparalytic keratitis propounded by Duke-Elder (1938) is based on Loewi's triple response, the corneal changes being due to an accumulation of metabolites which initiate tissue oedema and vasodilatation. The oedema then leads to impaired vitality, degeneration, and exfoliation of the corneal epithelium.

In this paper based on clinical observations made in the Eye Clinic of the Jaffna General Hospital and in the Leprosarium, Hendala, a tentative explanation is offered of the mode of action of tarsorrhaphy in neuroparalytic keratitis.

Material

Case 1. Excision of a left parotid tumour in a man aged 52 resulted in left facial palsy, and infiltration of the nasopharynx and orbit had led to left total ophthalmoplegia when the patient was first seen, and 4 months later neuroparalytic keratitis developed. The left ptosis did not prevent the onset of corneal ulceration. The patient refused a tarsorrhaphy and the left eye was lost owing to progressive ulceration of the cornea.

Case 2. A nasopharyngeal endothelioma on the right side in a man aged 48 led to total ophthalmoplegia and proptosis. The neuroparalytic keratitis which followed was cured by tarsorrhaphy.

Case 3. A nasopharyngeal tumour on the right side in a man aged 50 left him with an intact cornea, but neuroparalytic keratitis followed in 6 months' time. Tarsorrhaphy cured the keratitis, although ophthalmoplegia and proptosis were present when he was first seen.

Case 4. Congenital deficiency of the fifth nerve in a woman aged 48 led to bilateral neuroparalytic keratitis with an interval of a year between the onset in the two eyes. Tarsorrhaphy cured the condition.

Case 5. Generalized herpes with neuroparalytic keratitis of the right eye in a man aged 45 was not affected by plain bandaging, antihistamines, or cortisone with oxytetracycline, and wearing a contact lens (for 48 hours) made the condition worse. Finally tarsorrhaphy cured the ulceration.

Case 6. Trigeminal neuralgia in a man aged 45, and alcohol injection of the trigeminal

* Received for publication April 18, 1957.

ganglion was followed by neuroparalytic keratitis. He refused tarsorrhaphy, and when he was seen 10 years later the cornea had healed with a faint scar and there were traces of old iritis. The cornea as well as the lids were anaesthetic.

Cases 7, 8, and 9. Vesiculation of corneal grafts was healed by tarsorrhaphy.

Case 10. Fuchs's dystrophy in a woman aged 52 was relieved by tarsorrhaphy.

Case 11. Recurrent corneal erosion in the right eye in a man aged 50 was relieved by tarsorrhaphy.

Case 12. In leprous keratitis of the neuropathic type in the right eye in a man aged 55, the ulceration was healed by tarsorrhaphy.

Case 13. Acute keratoconus occurred in a boy aged 18; pressure bandaging (after the method of Terry and Chisholm, 1940) effected rapid relief of symptoms and the cone receded (Sivasubramaniam, Hoole, and Anandarajan, 1953).

Cases 1, 2, and 3 developed neuroparalytic keratitis in spite of coexisting ptosis. Case 1 refused tarsorrhaphy and lost the affected eye, but in the other two cases the eyes were saved by tarsorrhaphy.

In Case 5, antihistamines, cortisone with oxytetracycline, and splinting with a contact lens had no effect, but tarsorrhaphy eventually effected a cure.

Discussion

According to Paton (1926), involvement of the trigeminal nerve "seemingly in any part of its course" may lead to neuroparalytic keratitis. Paton believed that "there must be a central element in its causation" and that "protecting the eye by sewing the lids together invariably results in preventing the development of the condition or in the rapid cure of the condition if it has developed, which would seem to indicate almost equally definitely that there must be a peripheral element in its causation".

It must be recalled that the mere absence of nerve supply to the cornea is insufficient, as in cataract extraction and in penetrating keratoplasty neuroparalytic keratitis does not develop though corneal oedema may develop as a result of loss of sensation (Sarwar, 1953). It is striking too that the rest of the distribution of the fifth nerve does not exhibit any lesions in neuroparalytic keratitis and a tarsorrhaphy heals remarkably well despite anaesthesia of the skin. From the foregoing it appears that avascularity of the cornea renders it peculiarly susceptible to neuropathic keratitis. The peripheral element referred to by Paton is what Duke-Elder refers to as a loss of peripheral control over the metabolic activity of the cornea.

Basing the probable aetiology of neuroparalytic keratitis on Duke-Elder's theory, it is the writer's view that tarsorrhaphy acts as a splint to the cornea and supports it, while the movements of the globe behind such a splint helps to massage the cornea and expedite the removal of stagnant metabolites and thus reduce tissue oedema. A ptosed lid, while excluding trauma and desiccation, allows neuroparalytic keratitis to develop because it hangs limply like a curtain over the cornea and is not close enough to act as a splint. Even in the presence of proptosis, a ptosed lid does not function in

the manner described unless it is sewn to the lower lid, when the bulge of the eye makes the sewn lids a little taut and thus more effective even if the globe is immobile and the lids atonic.

Drawing a parallel from general medicine, supportive bandaging of an injured joint discourages oedema round it. Supportive bandaging of an eye may likewise reduce corneal oedema as in Case 13. In fact, early bandaging may avert an impending case of neuroparalytic keratitis, but this method has its limitations, because of the frequent changes necessitated.

A contact lens may afford protection to an anaesthetic cornea as after trigeminal ganglion injection (Ridley, 1954; Sarwar, 1953), though some doubt has been cast on its value in cases of established neuroparalytic keratitis (Williamson-Noble, 1953). If a contact lens is to act as a splint, as it must if it is to be of benefit in neuroparalytic keratitis, it must fit closely with only a hypothetical tear film between it and the cornea. The failure of the contact lens to produce satisfactory results in Case 5 is understandable though it has its place in exposure keratitis where the nerve supply to the cornea is intact.

Antihistamines including cortisone are probably ineffective because neuroparalytic keratitis is not an allergic manifestation.

The disappearance of vesiculation in cases of recurrent corneal erosion, herpes zoster of the cornea, and keratoplasty (Cases 7, 8, and 9) supports the view that the value of tarsorrhaphy lies in the splinting and massage which it provides. This would explain the observation that an adhesion only a few millimetres broad between the lids in a case of neuroparalytic keratitis may turn the tide in favour of the patient.

Segall (1955), discussing the problem of congenital deficiency of the fifth nerve, asserts that exposure seems to be an integral factor in the development of keratomalacia and neuroparalytic keratitis, since the ulceration occurs in the exposed areas of the cornea. It is the writer's view that the exposed areas are affected in neuroparalytic keratitis because of the lack of support of the cornea. Tarsorrhaphy may therefore be considered an emergency stop-gap measure to save not merely the cornea but the whole eye until such time as a readaptive mechanism comes into play, and a humoral control begins to operate in place of the nervous control.

My thanks are due to the Medical Officer-in-Charge, Government General Hospital, Jaffna, and to Dr. P. J. de Fonseka, Superintendent of the Leprosarium, Hendala, Ceylon, for granting me permission to publish the cases described in this paper.

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Brit. J. Ophthal. (1958) 42, 486.

CORNEAL "FLAP" INCISION FOR CATARACT OPERATION*†

BY

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One of the drawbacks of all classical incisions for cataract surgery has been the slow consolidation of the scar. According to Dunnington (1956) and to Ashton and Cook (1951), the first step in the healing of limbal wounds is taken by the swelling of the corneal lamellae, whereas the sclera plays a very passive role in the process. The retractability of the tissues then leads to a separation of the anterior from the posterior margin; a condition that is soon overcome anteriorly by epithelial proliferation, but which leaves a cleft posteriorly that has been demonstrated in monkeys up to 2 weeks after the operation. Gliedman and Karlson are said by Dunnington (1956) to have proved that the tensile strength of limbal wounds (sutured and without a conjunctival flap) amounts to only 6·5 per cent. of the normal (*i.e.* undisturbed limbal tissue) in the immediate post-operative period, to 15·4 per cent. of it on the ninth post-operative day, to 34 per cent. on the fourteenth day, and still only to 62 per cent. 6 months after the operation.

In the light of these findings, the necessity for an incision that would overcome this difficulty appears quite obvious. The technique to be described below is based on Wheeler's halving principle, in the sense that the superficial part of the incision does not coincide with the deeper part of the same. The incision is made wholly on corneal tissue on the assumption that such a wound, if properly sutured, would develop a greater tensile strength, thus preventing those untoward post-operative occurrences known to derive from sluggish limbal healing. These theoretical considerations have yet to withstand the test of experimental work, but over eighty cases of senile cataract operated on by this method seem to substantiate them.

Technique

After the usual pre-operative measures have been taken (pre-medication, good mydriasis, surface and retrobulbar anaesthesia, akinesis), the procedure below is followed:

(a) *Lid Separation Sutures.*—Two sutures to each eyelid are used by the author, anchored to the sheets by Pean forceps, but any other way of lid separation may be used. Should

* Received for publication August 21, 1957.
† Read before the Chilean Society of Ophthalmology, meeting of May 31, 1957.

the outer canthus noticeably press on the globe, a canthotomy should be made. Mistakenly, in the accompanying illustrations only one loop has been pictured for the lower lid.

(*b*) *Superior Rectus Fixation.*—This is done in the usual way. The muscle is taken by a long bridle suture from which a forceps can be left to hang back over the head of the patient.

(*c*) *Limbal Incision.*—The bulbar conjunctiva below the lower limbus is grasped with a scleral fixation forceps as for a von Graefe knife incision. An Atkinson sclerotome (Atkinson, 1956) is then used for making a limbal section that should slightly overpass the 3 and 9 o'clock meridians and be carried to a depth of approximately half the cornea. This section can also be made with any short-bladed knife, taking due care not to cut too deeply.

(*d*) *Preparation of a Corneal "Flap".*—The fixation of the globe at the lower limbal conjunctiva being maintained, the tip of a curved Gill corneal knife (or Barraquer's piriform knife) is engaged at 12 o'clock in the groove already made (Figs A and "a"), and is insinuated into the corneal parenchyma towards the centre of the cornea for about 1·5 mm., thus detaching a small superficial corneal "flap". By sliding the knife sideways towards the right side of the incision and by combining sliding and introduction on its left side, a corneal flap is easily detached that should reach from 8½ to 3½ all along the limbal incision. Its thickness should equal about half that of the cornea and its radial length should be about 1·5 mm.

(*e*) *Pre-placed Suture.*—A 6-0 black silk suture threaded on a 4-7 mm. needle, preferably of the Vogt-Barraquer type, is passed at 12 o'clock through the whole thickness of the flap near its edge and then at the corresponding point in the posterior corneal lip (Figs "b" and "D"). A toothed forceps, such as Barraquer's iridectomy and corneal forceps, must be used to secure a firm grasp of this edge. The author has found the knot made at the distal end of the suture (Gómez Márquez) a very efficient means of closing the wound quickly after removing the lens.

(*f*) *Penetration into the Anterior Chamber.*—While the assistant lifts the corneal flap and tilts it slightly away from the limbus, fixation of the globe is resumed as for steps "*c*" and "*d*", and the anterior chamber is penetrated with a small knife incision made close to the root of the flap, at say 1 to 1·5 mm. from the limbal groove and parallel to it (Fig. "B"). Transfixion with a von Graefe knife is very easily accomplished over such a small distance, and this is the author's choice, but a small keratome or a Gillette blade can also be used to advantage.

(*g*) *Enlargement of the Incision with Scissors.*—Castroviejo's corneal scissors held parallel to the plane of the cornea are used at this stage (Fig. "B"). The 3 and 9 o'clock meridians should be reached at from 0·5 to 1 mm. from the limbal groove in order to secure the offsetting of both incisions while at the same time providing for a wide berth for lens extraction. Care should be exercised at this stage not to include the iris in the bite of the scissors. Good illumination and the tilting off of the split part of the cornea by the surgeon himself will render this complication unlikely.

(*h*) *Iridotomy or Total Iridectomy.*—The corneal flap being slightly lifted, the surgeon grasps the iris (at 1 o'clock for the right eye and at 11 o'clock for the left eye) with a curved toothless forceps, brings it out at wound level, and makes a transverse nip in it, as close to its root as possible. A total iridectomy can be made should the case warrant it.

(*i*) *Extraction.*—This stage is carried out in the usual way, with either forceps or erisophake (Figs "C" and "c"). The incision under discussion does not hinder extrusion of the lens nor is it a deterrent to an extracapsular or loop type of extraction. Once this has

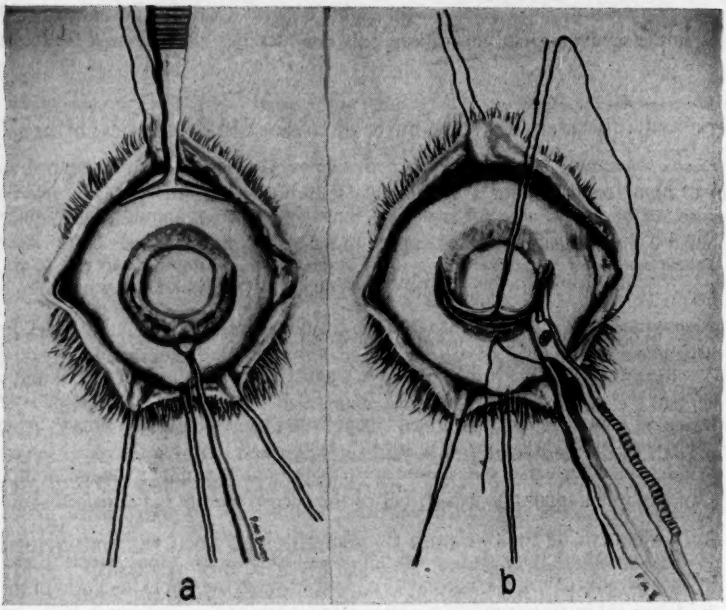

FIG. a.—The limbal groove has already been made and the Gill knife is beginning to split the cornea at 12 o'clock. Extent of splitting indicated by arrows.
FIG. b.—Enlargement of posterior incision with scissors. Notice insertion of pre-placed suture.

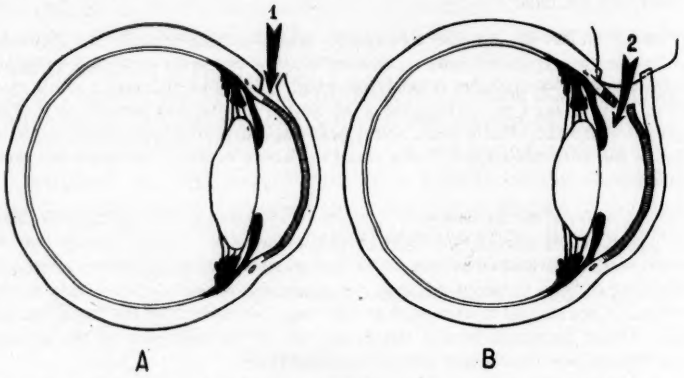

FIG. A.—Halfway limbal incision and splitting of the cornea (corneal "flap").
FIG. B.—Site of penetration into anterior chamber.

been accomplished, the suture is pulled taut, the Gómez Márquez knot serving as a stop. The iris edge, should it become entangled in the wound, can be easily freed by gently stroking it with a thin spatula.

(*j*) *Post-placed Sutures.*—Three sutures on the temporal side and another three on the nasal side of the pre-placed suture (Figs "D" and "d") should be used in order to ensure

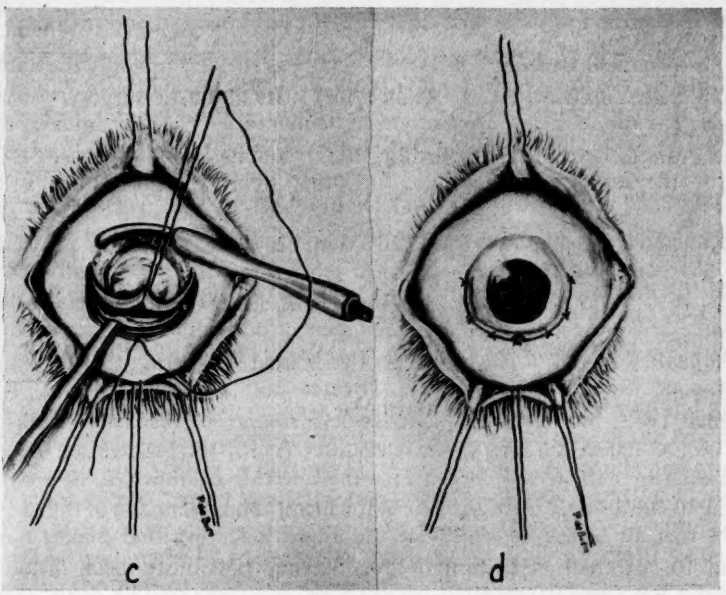

FIG. c.—Extraction. The incision gives a wide berth for manoeuvering the erisophake.
FIG. d.—Final result. Three post-placed stitches on either side of pre-placed suture. The eye is ready for air injection into the anterior chamber.

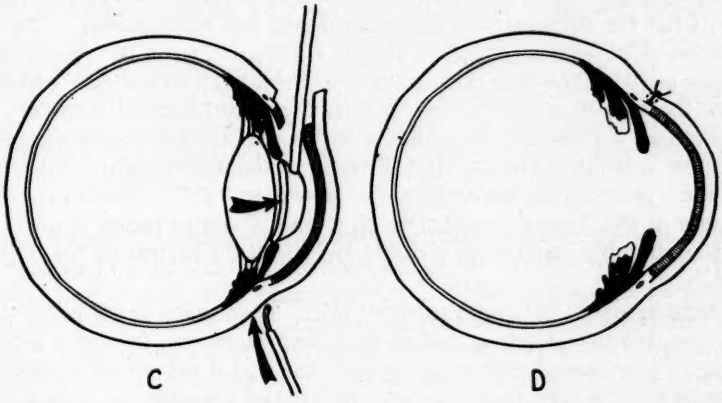

FIG. C.—Suction cup in anterior chamber.
FIG. D.—Final result. Note wide lamellar contacting surface and position of stitch.

a tight closure of the wound. Their technique is equal to that employed for the pre-placed suture.

Filling the anterior chamber with saline brings the operation to an end.

Discussion

(1) Incision and Sutures.—Wheeler (1936) was the first to apply the halving principle, used in carpentry, to obtain firmer scars in plastic surgery. Of late years it has found a new application in ocular surgery in the "mushroom" graft (Franceschetti, 1951; Sourdille, 1955), but has not been used, to the best of the author's knowledge, for other operative procedures in the eye.

Technically, the operative procedure is an easy one, as anyone can realize who has performed a lamellar corneal graft (Paufique, 1955; Paton, 1955; Tillett, 1956; Binder and Binder, 1956). The splitting of the cornea over such a small extension does not entail any dangers or difficulties provided adequate instruments are used. The limbal groove is used in several other techniques (Atkinson, 1956; McLean, 1940; Olivares and Rojas Echeverría, 1953; Lindner, 1942; Stocker, 1956), and its performance by means of Atkinson's sclerotome makes for a very neat incision. As for the penetration through the posterior half of the cornea into the anterior chamber, it is eased by having to cut through only one half of the membrane. The site of the incision leaves free all of the structures of the angle, a feature that makes it very useful for cataract extraction in eyes having previously been fistulized. Indeed a few cases in this predicament have been successfully dealt with by this technique.

With small, sharp needles and a fine, firm-toothed forceps these sutures are among the easiest and least cumbersome in this type of surgery. The first suture has the advantages of being pre-placed and appositional. The usual precaution of not reaching too deep is eliminated, since the needle should engage anyway the whole thickness of the corneal flap on one side, and should enter the bottom of the furrow made by the sclerotome on the other. The post-placed stitches are made quite safely as they are not closing the anterior chamber directly. The iris root obviously cannot be caught in the sutures since it is protected by the deep corneal layer (Fig. "D"). The firm and tight closure of the wound provided by this type of suture makes it possible to leave routinely a monocular dressing and to get the patient up the following day.

Dunnington (1951) and Verdaguer (1955) have made histological studies proving that the sloughing caused by a deep suture tightly tied is a definite cause of a weakened spot in the wound, and that if the slough is sufficiently deep or if the suture penetrates into the anterior chamber the stage is set for aqueous escape. Vail (1935) stated that among the precursors of epithelialization of the anterior chamber the most important are: a slowly healing wound, delayed formation of the chamber, an aqueous leakage, and fistulization of the wound. The fact that this is a two-step incision with a relatively large surface of lamellar adhesion, as well as the particularly safe disposition of the non-penetrating sutures, should make for a very low incidence of all

the forementioned occurrences, and indeed they have not presented themselves in the admittedly small series of operations done by this method.

Another advantage of this type of wound closure lies in the fact that the removal of sutures (on the twelfth day) is far less fraught with danger of opening or emptying the anterior chamber, because the deep part of the incision is offset from the sutures.

In the absence of rubeosis or a haemorrhagic diathesis, post-operative hyphaema in cataract surgery is usually explained by the rupture of newly-formed vessels spanning the corneo-scleral section (Bellows, 1956). The present author agrees with this theory, since his incision, done entirely in avascular tissue, has avoided such complications in the eighty-odd cases operated on both by himself and by Dr. Miguel Millán (personal communication).

In none of the operated cases in which seven sutures were used did an iris prolapse occur. This complication did happen in one case at the beginning of the series, at 9 o'clock in the limbus; this was due to an insufficient number of stitches and to having made the two incisions coincide on that meridian. The prolapse having been discovered on the following day, an immediate reduction was made by means of a thin spatula, and a corneal stitch was inserted; the patient was subsequently discharged with an almost round pupil. This case proved the importance of offsetting the incisions all along the wound as well as of placing three sutures on either side of the first incision at 12 o'clock. Once these precautions were taken no more complications were seen, which probably indicates that this system of incision and sutures should be the method of choice in unruly or agitated patients as well as in linear extraction for congenital cataracts.

For those who prefer a buried silk or catgut suture, the making of the limbal groove should be preceded by the fashioning of a limbal-based small conjunctival flap. In the opinion of the author, however, this merely complicates matters unnecessarily.

(2) Extraction.—Neither intra-capsular nor extra-capsular extractions have been hindered by the fact that the deep part of the incision does not actually reach the limbus itself at the 3 and 9 o'clock meridians, and that about 1·5 mm. of Descemet's membrane plus some corneal parenchyma are left at the upper limbus. This should not be surprising if one considers that the deep incision measures about 10 mm. from 3 to 9 o'clock and is capable of gaping anterio-posteriorly far more than the 3·5 mm. maximum thickness of the lens. Depression of the thinned corneal remnant at 12 o'clock has never failed to bring out even a large nucleus in the course of an extra-capsular extraction. Subluxated lenses have been extracted with the loop by first insinuating its tip backwards at 12 o'clock, sliding it between the lens and what remains of the upper cornea (a total iridectomy should have been made before using the loop), and proceeding afterwards in the usual way.

Vitreous loss seems to happen less frequently in the corneal flap operation than in other types of cataract surgery. Preservation of a corneal diaphragm may account for this feature, which remains, however, to be confirmed by a larger series. The event of this complication does not call for a totalization of the iridotomy. The pupil may suffer a slight deformation but has never been seen to draw upwards in the cases that sustained vitreous loss.

(3) **Contraindications.**—Eyes with very flat or non-existent anterior chambers should not be operated on by this method, as in them there would be a definite danger of incarceration of the iris between the lips of the deeper corneal section. Cases of Fuchs's endothelial dystrophy may be made worse by a purely corneal section, but no dystrophies have appeared post-operatively in the author's series.

Summary

A cataract incision is presented, based on Wheeler's halving principle. It is performed by dissecting a corneal "flap", limited at the limbus by a superficial incision reaching into the anterior half of the corneal thickness and centrally by a penetrating incision through its posterior half. The latter is made 1·5 mm. central to the anterior half of the corneal thickness at 12 o'clock, and from 0·5 to 1 mm. from it at the 3 and 9 o'clock meridians. This technique includes one pre-placed and six post-placed corneal sutures which are safely non-penetrating and separated from the deeper incision by a distance of 1·5 mm. on the 12 o'clock meridian.

The main advantages of this method derive from a quick and firm closure of a corneal wound made in avascular tissue with a large surface of lamellar contact: lack of iris prolapse, flattening of the anterior chamber, and hyphaema. Theoretically, it should prevent some forms of aphakic glaucoma and epithelialization of the anterior chamber. It also provides a new way of dealing with cataracts in eyes already operated on for glaucoma.

The tight closure of the anterior chamber that it affords makes it possible to leave a monocular bandage and to get the patient up on the following day. Removal of sutures is far less dangerous than in other procedures because of the separation existing between the point of their insertion and the line of penetration into the anterior chamber.

Extreme flattening of the anterior chamber pre-operatively and Fuchs's endothelial dystrophy would seem to be the only contraindications to this specific type of incision.

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Brit. J. Ophthal. (1958) **42**, 494.

EXPERIMENTAL ELECTROCHEMICAL REMOVAL OF COPPER SPLINTERS FROM THE ISOLATED VITREOUS*

BY

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THE penetration of a splinter of copper or copper alloy into the eye always presents a serious problem. If the metal, especially in a relatively pure state, is left in the eye, it is bound to produce a heavy reaction in the form of acute iridocyclitis, violent uveitis, or vitreous abscess with all the sequelae of these conditions. Copper alloys containing less than 85 per cent. of copper usually cause a less severe reaction, but then chalcosis may develop, a condition which in most cases leads to a gradual loss of vision, although its course depends on the individual tolerance of the eye to the metal.

The most reasonable though not the easiest course of action is to remove the copper splinter mechanically, using one of the methods devised for the removal of non-magnetic foreign bodies. These methods, though constantly being improved, are still far from perfect. Both the older instruments, such as the Desmarres-Luer forceps, and the more recent ones, such as the Holth forceps or the endoscopic appliances, may prove inadequate, and the only result of the operation will be a severe additional trauma. The risk is sometimes so grave that all idea of operative treatment must be abandoned.

Some investigators have endeavoured to coat the splinter with a deposit of a neutral metal, such as gold, or to produce a magnetic alloy with iron (Duke-Elder, 1954). Müller (1931) and Habig, Lumen, and Snacken (1951) tried to convert copper into a harmless compound by the local or general application of sodium thiosulphate or sodium hyposulphite. Such attempts, as well as those making use of the property of BAL to form an insoluble compound with copper (Newell, Cooper, and Farmer, 1949), have been failures from the clinical point of view. The idea of introducing in the region of the foreign body an amalgam to which the latter would then attach itself firmly enough to be withdrawn (Wölflina, 1946) was no more successful than that of removing a non-magnetic foreign body by means of electro-magnetic forces (Endt and Ten Doesschate, 1949).

Nevertheless, the idea of a non-instrumental method of removing a copper foreign body from the eye is so attractive that it should not be given up before all possibilities have been investigated.

The purpose of our experiments was to examine the possibility of the

* Received for publication April 23, 1957.

electrochemical removal of copper from the isolated vitreous. In this connexion two questions had to be answered:

(1) Is it possible to dissolve a small copper splinter electrolytically, using the vitreous as electrolyte, and what is the efficiency of this process in experimental conditions?

(2) To what extent is it possible to deposit the previously dissolved metal on the cathode, and what is the efficiency of this process?

Methods

A concavity ground in a glass plate was filled with fresh bovine vitreous. The capacity of the concavity was about 6 ml. In the vitreous, which was to play the part of an electrolyte, copper splinters were placed, their weight ranging from 1·6 to 5·4 mg., and their diameter from 2 to 3 mm. Then the active electrode (anode) was so placed in the vitreous that it came into contact with the copper splinter. Near the edge of the vessel, 15 to 20 mm. away from the splinter, a subsidiary electrode was immersed in the vitreous. A platinum needle was used as the working electrode; in some of our experiments this was coated with an insulating material, only a point 0·5 mm. long being left bare. When the direction of the electric current was to be changed, this electrode was replaced by a non-insulated platinum needle. As the subsidiary electrode, a platinum plate was used, measuring 2 × 5 mm., and mounted in glass. The current was supplied by a small dry battery with branches from the separate cells so that a tension of 1·5, 3·0, or 4·5 V could be chosen. The intensity of the current was measured with a milliampèremeter, the sensitivity of which was 7 mm./1 mA. To facilitate the manipulation of the electrodes, a special stand was built with movable clamps for the electrodes and a magnifying glass as an aid in observing the electrolytic process.

After the electrodes had been placed in position, the electric circuit was closed. If the contact between the anode and the copper splinter was maintained, the needle of the galvanometer moved from its initial position. At the same time hydrogen bubbles began to appear round the subsidiary electrode, and a few seconds later a greenish stain could be seen in the vitreous round the anode, which pointed to a gradual dissolution of the copper. The time and the galvanometer readings were taken. When the current intensity began to fall and when it was found that this was due to the complete dissolution of the copper, the time and the final current intensity were again taken, and then the direction of the current was reversed, the working electrode becoming the cathode and *vice versa*. The immediate effect of this, as observed on the galvanometer, was an abrupt rise in the current intensity, which was ten times higher than that of the dissolving current. At that moment the copper which had been dissolved in the vitreous began rapid deposition on the platinum needle (cathode). The time and current intensity were recorded and, when the latter fell to a constant level, a quantity of

current two or three times higher than the theoretical current was passed and the experiment ended. The cathode coated with the deposited copper was rinsed first in concentrated nitric acid and then in water; this served as a preparation for the quantitative determination of the copper obtained from the vitreous by means of electrolysis.

Results of Investigations

Altogether eleven experimental attempts at the electrolytic (anodic) dissolution of copper splinters were carried out. The results of these experiments are presented in Table I.

TABLE I

RESULTS OF ANODIC DISSOLUTION OF COPPER

Experiment No.	Mass of Splinter (mg.)	Volts	mA		Time (min.)	Current Efficiency (per cent)	Anode
			Range	Mean			
1	5·2	4·5	15–6	9	80	36	Non-insulated
2	4·8	4·5	15–5	8	80	38	,,
3	5·2	3·0	10–4	6	100	44	,,
4	3·6	3·0	6–3	4·5	60	67	Insulated
5	1·4	3·0	6–2	4	20	88	,,
6	5·1	4·5	11–5	7	90	41	,,
7	5·4	4·5	12–3	6	100	45	,,
8	2·3	4·5	13–5	8	30	48	Non-insulated
9	1·6	4·5	12–4	7	20	58	,,
10	1·9	4·5	10–4	6	30	53	,,
11	2·1	4·5	7–3	5	35	61	,,

The analysis of current efficiency based on the data collected in Table I shows that its value changes considerably under the influence of several factors. The most important is the mass of the foreign body. This is natural if we consider that only some, especially the chlorine, anions of the vitreous take part in the dissolution of copper. Near the anode their number is comparatively small and decreases steadily as electrolysis continues, so that towards the end of the experiment the function of carrying electric charges is taken over by the hydroxyl ions, which have no influence on the process of dissolution of copper, and only produce gaseous oxygen at the anode.

It was found that when the anode was covered with an insulating coat, only its point being left bare, the efficiency of the dissolving process increased considerably. This is due to the fact that the anions which leave the non-insulated platinum anode above the point of contact take no part in dissolution. It was possible to prove that the dissolution of copper under the influence of a passing electric current went on most actively at the point of contact between the two metals, where the current density is highest. The curves shown in Fig. 1 (opposite) show the evident relation between the mass of the copper splinter and the efficiency of the dissolution current for both the insulated (continuous line) and the non-insulated (dotted line) electrodes.

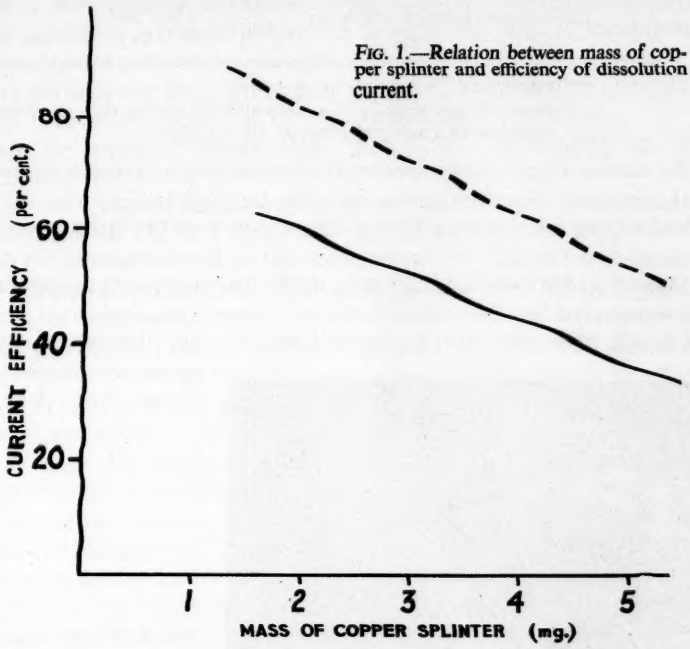

FIG. 1.—Relation between mass of copper splinter and efficiency of dissolution current.

The mechanism of the anodic dissolution of a copper splinter may be explained in the following way. The splinter receives a positive electric charge from the working electrode. This leads to the depolarization of the vitreous anions which at that moment are very close to the anode. If among these depolarized anions there are some which can form chemical compounds with the metal of the anode, in our case with copper, the dissolution of the anode will take place and part of the anodic work of the current will be used for sending cations coming from the dissolved anode into the solution. The ratio of that part of the work of the current used for dissolving the anode to the whole of the work performed by the current, expressed in percentage, is called in this case the dissolution current efficiency. It is expressed by the formula:

$$\text{Dissolution current efficiency} = \frac{100\,Cr}{C} \text{ per cent.} \quad . \quad . \quad (1)$$

where Cr = amount of electricity (expressed in coulombs) used for the dissolution of the anode,

C = total amount of electricity (in coulombs) passed through the electrolyte.

When coulombs are expressed in milliampère-minutes and the part of the work of the current used for dissolving copper is given in milligrammes of dissolved copper, the formula is as follows:

$$\text{Dissolution current efficiency} = \frac{100\,a}{0{\cdot}0198\,it} \text{ per cent.} \quad . \quad . \quad (2)$$

where a = amount of copper (in milligrammes) dissolved from the anode in t minutes,
i = mean current intensity (in milliampères) during the experiment,
t = duration of dissolution current (in minutes).

In the second stage of the experiment we attempted to remove copper ions from the vitreous. For this purpose the cathodic precipitation (removal) of the dissolved copper was applied. Deposition of copper on the cathode took place immediately after dissolution on simply reversing the direction of the current. If an insulated electrode had been used in the first stage of the experiment, it was now replaced by a non-insulated one. Copper was deposited along the whole length of the platinum needle submerged in the vitreous and could be removed together with the electrode (Fig. 2).

FIG. 2.—Copper deposited on platinum needle and removed together with electrode.

The amount of copper deposited on the cathode was determined iodometrically; when a microburette was used, the margin of error in this method was only 5 per cent. The results of cathodic deposition of copper are given in Table II. The efficiency of the deposition current is seen to be much lower

TABLE II

RESULTS OF CATHODIC PRECIPITATION OF COPPER

Experiment No.	mA		Time (min.)	Mass of Precipitated Copper (mg.)	Current Efficiency (percent.)	Quantity of Deposited Copper (percent.)
	Range	Mean				
2	60–4	15	45	2·0	15	42
3	52–5	12	45	2·5	23	48
4	52–4	14	55	2·0	13	56
5	48–4	16	20	0·9	14	66
6	55–5	12	50	2·6	22	51
7	58–5	18	40	3·3	26	61
8	52–4	15	20	1·7	29	76
9	50–4	18	20	1·1	15	71
10	46–4	12	20	1·4	29	75
11	46–4	12	20	1·7	36	79

than that of the dissolution current. The total percentage of copper deposited on the cathode is in inverse proportion to the initial mass of the splinter and, therefore, to the duration of electrolysis. This might suggest that when electrolysis lasts too long, the copper passes into irreversible complex compounds from which it cannot be precipitated electrochemically.

When the copper is dissolved and the current discontinued, the vitreous surrounding the anode presents a complex mixture of various chemical compounds. The dissolved copper passes into the solution first as a chloride; some of the chlorine ions which have lost their electric charge on the working electrode are given off in the form of gaseous chlorine. This active element combines freely with the surrounding proteins, giving various products of chlorination. The atomic oxygen which forms at the anode, especially in the later stage of dissolution, partly takes the free, gaseous form, and partly reacts with the free chlorine giving subchlorous acid, the remainder probably exerting an oxygenating effect on the organic compounds which surround the anode. The copper chloride, which initially passes into the vitreous, may also combine with the proteins to form copper albuminates, or may undergo hydrolysis in the vitreous.

In view of the purpose of our investigations it was particularly important to find out what quantity of copper remains in the vitreous in the form of ions, and what quantity enters into stable complex compounds or assumes an insoluble form, as the metal is only liable to electrolytic precipitation on the cathode in the ion form. In the conditions of our experiment it was possible to remove by means of cathodic deposition up to 79 per cent. of the copper dissolved in the vitreous. The residue of the copper probably passed into a non-ionized form and could not be deposited on the cathode independently of the duration of the electrolytic process.

The results of our investigations show that it is is possible to obtain anodic dissolution of a copper splinter in the isolated vitreous, as well as cathodic deposition of a considerable part of the splinter (up to 79 per cent.), and thus to remove the metal as a thin coat covering the cathode. It would be too much to expect that these experiments might influence the clinical procedure of removing copper foreign bodies, but further investigations, carried out if possible on experimental animals, may assist the practical application of this idea.

Summary

The anodic dissolution of a copper splinter placed in isolated bovine vitreous was investigated using insulated and non-insulated electrodes. The current efficiency of the process was calculated, and the relation between the current efficiency and the mass of the splinter and the kind of electrode was shown graphically.

In the second part of the experiment the possibility of the cathodic precipitation of the dissolved copper was investigated and the current efficiency of this process was also calculated.

The results of the investigations show that it is possible to dissolve and precipitate electrochemically up to 79 per cent. of copper particle placed in an isolated bovine vitreous.

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Brit. J. Ophthal. (1958) **42**, 501.

OCULAR MANIFESTATIONS IN IDIOPATHIC HYPERLIPAEMIA AND XANTHOMATOSIS*

BY

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THE accumulation of neutral fat in the blood to such an extent as to produce turbidity of the serum is termed hyperlipaemia. This may occur physiologically after a fatty meal, secondarily to diabetes mellitus, the nephrotic syndrome and glycogen storage disease, or idiopathically.

The literature on idiopathic hyperlipaemia has recently been reviewed by Lever, Smith, and Hurley (1954), who collected 41 cases from the literature, to which they added seven of their own. The condition is not infrequently familial and may begin in childhood; in two-thirds of the cases reviewed by Lever and others (1954), the disease was discovered before the age of 30. It may be accompanied by cutaneous xanthomata; the histological features of these eruptive xanthomata have been considered by Thannhauser and Magendantz (1938). Xanthomata of the tendons are also encountered. Episodes of abdominal pain, probably from pancreatitis, are common, and coronary artery disease may occur. The spleen and liver are frequently enlarged and glycosuria may be found.

With respect to the blood lipids, cases of xanthomatosis exhibit two extreme types:

(1) Hypercholesteraemic xanthomatosis, where there is a clear serum, a great increase in cholesterol with little increase in neutral fat, and an electrophoretic lipoprotein pattern that differs from the normal only in the occurrence of a very high β-lipoprotein fraction;

(2) Hyperlipaemic xanthomatosis, where the serum is grossly lipaemic and there is an electrophoretic pattern in which the β-lipoprotein is submerged in a mass of other lipid; the hypercholesteraemia is moderate, and Thannhauser (1950) suggests that it is secondary to the hyperlipaemia.

The two conditions do not, however, appear necessarily to be of unrelated aetiology, since Smith (1956), in a study of thirteen cases of xanthomatosis, five of which were examples of idiopathic hyperlipaemia, found two that appeared to change in type during a follow-up period of about a year. One changed strikingly from an apparently pure hypercholesteraemia to a moderately severe hyperlipaemia and the other lost the moderate degree of hyperlipaemia that was present when he was first seen.

* Received for publication July, 29, 1957.

† Formerly House Physician, West End Hospital for Neurology and Neurosurgery.

The present case, which falls into the latter category, is unusual because of its presentation with visual failure and also in the occurrence of retinal xanthomata; it is reported for this reason, together with some observations on the nature of the biochemical disturbance.

Case Report

A 58-year-old woman was admitted to the West End Hospital for Neurology and Neurosurgery in July, 1954; 5 months before her admission she had discovered, on accidentally covering the left eye, that the vision in the right eye was blurred. Subsequently, the vision had improved, but central blurring had remained.

Her previous medical history included a cholecystectomy in 1953, because of gall stones. Symptoms referable to this condition dated back for 10 to 15 years. Since the age of 30, recurrent cutaneous papules, which were described as containing yellowish material, had been present, mainly over the elbows, knees, and outer sides of the feet. It is of interest that the patient had observed that these had subsided when she had been placed on a low fat diet because of her gall bladder disease.

The patient had two daughters and a son. She had an elder sister and a younger half-sister; another half-sister had died during childhood from tuberculosis. The patient knew of no relatives with any similar disorder and there was no known consanguinuity. Of those just mentioned, it was only possible to examine the blood of the patient's two daughters; neither showed a macroscopic lipaemia.

Examination.—The visual acuity was 6/60 in the right eye and 6/12 in the left (with glasses). The peripheral fields were full in both eyes, but in the right there was a large central scotoma (Fig. 1). The right fundus showed oedema of the macular region,

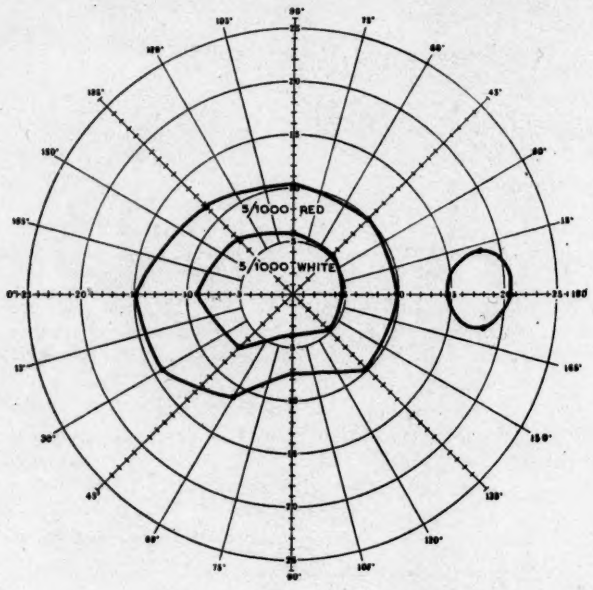

FIG. 1.—Bjerrum screen chart indicating scotoma.

together with old haemorrhages in this area. Of particular interest was the presence of fairly numerous small, yellowish-white, clearly defined deposits, distributed especially along the lines of the vessels; these were considered to represent minute xanthomata. The left fundus also showed these deposits but was otherwise normal (Fig. 2). Lipaemia retinalis was not observed. Vertical nystagmus and horizontal nystagmus on lateral deviation of the eyes to both sides was present, but there were no other abnormal neurological signs.

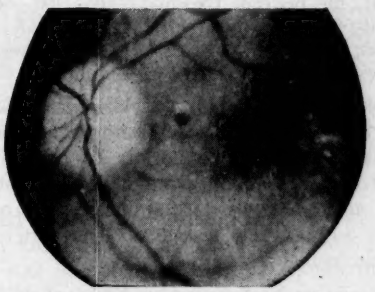

FIG. 2.—Retinal photograph of case described in text.

On the lateral aspects of both feet were clusters of reddish papules with a central yellow area, the largest being approximately 1 cm. in diameter. At other times, linear streaks of small xanthomata were noted on the shins, presumably in the line of scratches with the nails.

The spleen was moderately enlarged and the liver edge palpable 6–7 cm. below the costal margin. Otherwise examination yielded nothing of significance except for a blood pressure of 180/100.

Macroscopic lipaemia was discovered on taking a sample of venous blood.

Progress.—After investigation, the patient was discharged on a low fat diet and was re-admitted for further assessment after periods of 3 months and 1 year. On her last admission, the findings were essentially unaltered, save for the fact that the macular region on the right now showed pigmented degenerative change. The visual acuity was unaltered and there was no significant change in the size of the scotoma. The size of the liver and spleen was relatively unchanged, but the patient admitted that she had not kept rigidly to the diet.

Laboratory Investigations

Haematological Findings

No abnormality of the peripheral blood was detected at any time. Biopsy of the sternal marrow showed foamy vacuolation of the reticulum cells. These were not obviously increased in number, but some were so swollen with droplets that they reached sizes of up to 65μ in diameter. Certain cells resembling monocytes also showed a similar vacuolation of their cytoplasm in lesser degree. The marrow was otherwise normal.

Biochemical Findings

The serum lipoproteins were examined at two periods; four separate serum samples were examined during July, 1954, and three samples during October and November, 1955. There was possibly some very slight improvement at the latter

period, but in all samples examined the lipids were grossly abnormal, the fresh serum being very milky and the lipoprotein emulsion unstable, separating into a layer of "cream" and a more or less clear infranatant on standing.

Analytical Data

Total cholesterol 367 mg. per cent. (Normal range—250 ± 50 mg. per cent.)
Lipid phosphorus 16·5 mg. per cent.
Phospholipid 412 mg. per cent. (Normal range—300 ± 50 mg. per cent.)
Cholesterol/phospholipid = 0·89 (Normal range −0·8 ± 0·3)
Total petroleum ether soluble material 3·2 g. per cent. (Normal range very variable; does not usually exceed 1 g. per cent.)

Electrophoretic Lipoprotein Pattern.—Paper electrophoretic patterns prepared by the method of Dangerfield and Smith (1955a) showed a mass of lipid on the origin and in the "trail" extending from the origin to the β-lipoprotein. A dense pre-β-lipid component extended to the front of the α_2-globulin, and the β-lipoprotein, which was of approximately normal density, was only just discernible in this mass of fat. The α-lipoprotein was low. The pattern is compared with a normal lipoprotein pattern in Fig. 3.

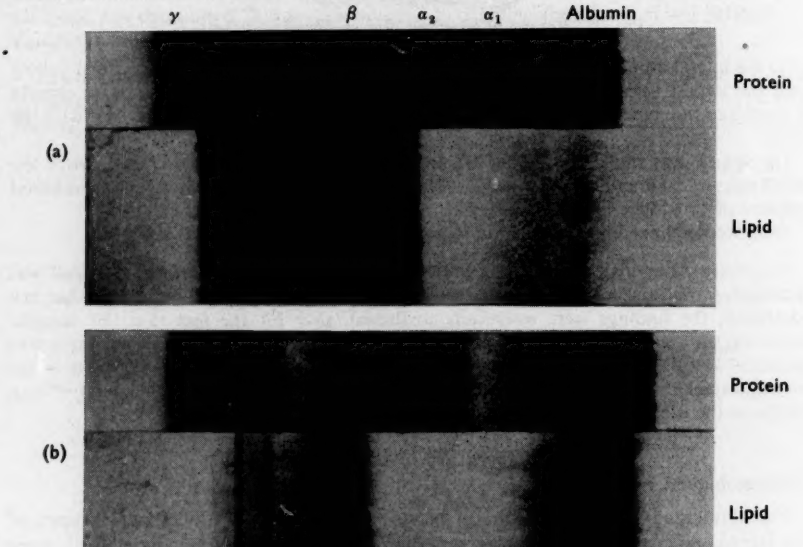

FIG. 3.—Electrophoretic patterns of serum dyed for protein and lipid.
(*a*) The patient
(*b*) A normal woman

Response to Intravenous Heparin.—Intravenous injection of 10,000 u. heparin temporarily stabilized the lipid emulsion and produced acceleration of the lipids on paper electrophoresis (Dangerfield and Smith, 1955b). Treatment with 10,000 u.

heparin three times weekly for 3 weeks had no permanent effect on the lipoproteins.

It has been shown that in the majority of subjects acceleration of the β-lipoprotein occurs after the intravenous injection of as little as 25 u. heparin (Smith and Dangerfield, 1955). The acceleration is increased by fat feeding and subjects who do not respond when fasting generally respond after fat feeding. This patient gave no response to 25 u. heparin either fasting or after a fatty meal, but responded to 100 u. heparin. This presumably indicates some degree of heparin resistance; of two other cases of idiopathic hyperlipaemia tested with 25 u. heparin, one responded normally and the other gave no response after feeding.

Glucose Tolerance.—An oral glucose tolerance test yielded normal results. Glycosuria was not present at any time.

Liver Biopsy (Dr. F. D. Bosanquet)

The liver cells appeared normal; they contained a quantity of glycogen, but this was not considered to be excessive. There was no suggestion of cirrhosis, and the amount of fat present was not obviously increased. In the portion obtained, therefore, there was no evidence of abnormal lipid or glycogen storage and no nests of xanthoma cells, such as described by Thannhauser and Magendantz (1938), were seen. The size of the biopsy specimen, however, was not large.

Other Investigations

A chest *x* ray showed moderate left ventricular enlargement, and an electrocardiogram revealed left axis deviation but no other abnormality. The cerebrospinal fluid was normal.

Comment

Ocular changes in idiopathic hyperlipaemia, with the exception of palpebral xanthomata, arcus lipoides corneae, and lipaemia retinalis, have been recorded only rarely. In the patient reported by Frank and Levitt (1951), xanthomatous deposits were visible on the iris of both eyes. The present case resembles that of Lewis (1950), in which intra-ocular deposits occurred in association with xanthomatous biliary cirrhosis. The patient presented with impaired vision in one eye and examination of the fundus revealed scattered yellowish areas of varying size involving all parts of the fundus. These were situated deep to the retina and at one point had produced retinal detachment. Areas of oedema of the retina with haemorrhages were noted. In the present case, the deposits, apart from the lesion involving the right macula, gave the impression of being more superficially situated.

The blood lipid picture is typical of essential hyperlipaemia, but an unusual finding in this patient is the instability of the lipid emulsion, particularly in view of the very low value of the cholesterol/phospholipid ratio. It is often loosely assumed that phospholipid helps to maintain the other lipids in solution or suspension, but this case suggests that the relationship is very complex because even the ratio of total serum lipid/phospholipid is markedly

lower than in a comparable case with over 5 per cent. total serum lipid and a perfectly stable lipid emulsion.

Its well-known lipaemia-clearing action provides the rationale for treatment with heparin, and in a few cases it has proved effective (personal communications), but in this case it is possible that both the dosage and the period of treatment were inadequate. The stabilization of the lipid emulsion after heparin injection coupled with some degree of heparin resistance does, however, suggest the possibility that heparin metabolism is in some way connected with the aetiology of the condition.

We wish to thank Dr. B. G. Parsons-Smith for permission to publish details of this case and, with Dr. Michael Kremer, for helpful advice in the preparation of this report. The retinal photograph was prepared by the photographic Department of the Institute of Ophthalmology.

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Brit. J. Ophthal. (1958) **42**, 507.

CORRESPONDENCE

REMOVAL OF CORNEO-SCLERAL SUTURES

To the Editorial Committee of the BRITISH JOURNAL OF OPHTHALMOLOGY

SIRS,—

"Ther nis no newe gyse that it nas old". *Knight's Tale*, l. 1267 (Chaucer *c.* 1380).

"Il n'y a de nouveau que ce qui a vieilli". *French Proverb.*

It is, of course, important that Mr. P. Wilson (*Brit. J. Ophthal.* (1958), **42**, 248) has brought to the attention of your readers the technique of removing corneo-scleral sutures with the clean tangential cut of a Bard-Parker knife, the suture being lightly held by fine plane forceps. However, this practice is not new, for it has been done at Moorfields, at least in the clinic which I serve, for over 10 years. Also I used this method in the 1939–45 war for removing silk sutures from the edges of free skin grafts. I do not remember its origin, which I think must have been before 1939.

Mr. Wilson reports a mishap when toothed forceps were used. Indeed it is a first surgical principle that sutures and the edges of a conjunctival incision should be held only by plane forceps and never by toothed forceps. It is preferable for the ends of these forceps to be blocked so that coaptation is sure and for the terminal 2 mm. to be faintly hatched to enhance the grasp of a mucous-soaked 6/0 silk suture.

I think that in spite of Mr. Wilson's concern about the anxiety induced in a patient returning to the operating theatre it is desirable to remove corneo-scleral sutures in the quiet of a well-disciplined theatre and not in a general ward as Mr. Wilson suggests, where there are so many distractions and noises.

In the past, two serious disasters have occurred when this has been done in the wards at Moorfields and none in the operating theatre.

Yours faithfully,

H. B. STALLARD.

82 HARLEY STREET,
LONDON, W.1.
May 14, 1958.

BOOK REVIEWS

The Art of Clinical Refraction. By THEODORE H. WHITTINGTON. 1958. Pp. 286, 78 figs. Oxford University Press. (30*s.*)

This small book concerns itself not with the science but with the art of refraction; and the tale is told with almost religious zeal, so that "the priceless possession" of sight, "next in value to life itself", may be freely available, with "the added comforts of happy binocular vision". Mathematics and optical formulae are absent. The mechanism of retinoscopy is said to be a dull affair—it is, indeed, somewhat difficult to understand in its non-mathematical representation in these pages—and the author's aim is to clothe these dry bones with human interest and add zest to routine. To some extent he succeeds in this; and the book forms interesting reading, particularly with its insistence on a full explanation to the patient of the whys and wherefores of optical aids; but unfortunately it adds little new to a subject on which much has already been written.

Clinical Neurosurgery, Vol. 4. The Proceedings of the Congress of Neurological Surgeons, Chicago, 1956. Pp. x+140. Baillière, Tindall and Cox, London. (56*s.*)

Volume 4 of Clinical Neurosurgery contains the edited proceedings of the sixth annual meeting of the Congress of Neurological Surgeons held in Chicago during November, 1956. It is dedicated to Dr. Wilder G. Penfield, who was the honoured guest and who read a fascinating paper entitled "Thoughts on the Function of the Temporal Cortex". The other contributions were devoted to the neurological aspects of the pituitary region and the most interesting for the ophthalmologist was Wilbur Rucker's paper on "Ocular Manifestations of Pituitary Tumours", including twelve case reports to demonstrate the various ocular findings. The field changes receive particular attention, there is an excellent assessment of the significance of disc pallor, ocular palsies are described, and the differential diagnosis of chiasmal lesions concludes the chapter.

The radiological aspects of lesions about the sella turcica are described by J. D. Camp, the operative approach to the pituitary gland by J. Grafton Love, the surgical and irradiation treatment of pituitary adenomas by G. Horrax, hypophysectomy for cancer of the breast by B. S. Ray, and the physiological effects of hypophysectomy by O. H. Pearson. At the end of each chapter there is a question and answer series which enlivens the text and keeps the expert's feet well planted on the ground. The book is small and reads easily: there is no attempt to be comprehensive either in the papers or in the bibliography and this is a pleasant feature.

Clinical Neuro-Ophthalmology. By F. B. WALSH. 2nd edition, 1957. Pp. xvi+1,294, 441 figs. Baillière, Tindall and Cox, London. (11 *guineas*)

The second edition of Walsh's "Clinical Neuro-Ophthalmology" is much larger than its predecessor of 10 years ago. There are 1,294 pages, including the index, and by dint of arranging three columns on a page, the work has been kept in a single volume. It is of course a reference book and the reviewer does not claim to have read it from cover to cover, but he has used the first edition in his daily work over the last decade and the present edition for a period of 3 months. The index is excellent and guides the perplexed clinician quickly to the answers he seeks.

Although this is primarily a work of reference, it is possible to open the book at any page and read a short chapter on some aspect of a neuro-ophthalmological problem. The text is divided into relatively small sections, each complete in itself; they are headed clearly and have their separate lists of references. A special feature of the book is the publication of case reports; they indicate to the reader the variability of symptomatology even where the underlying pathological process is constant, and they are most helpful in describing the symptoms and signs chronologically so as to show the changing pattern which a disease process often displays. Another most welcome feature is the interpolation of the author's own observations. He sums up the literature and condenses opinion on unsettled points, but does not shrink from adding what has been his own experience.

Neuro-ophthalmology is a kind of no-man's land. In Great Britain, as far as the reviewer is aware, there is no physician or ophthalmologist whose practice is entirely limited to neuro-ophthalmology. Many of the cases require investigation of the visual fields followed by careful assessment of their significance, together with a detailed clinical, radiological, and biochemical examination of the nervous system. The ophthalmologist can, by reason of his opportunity to study fundi in large numbers, offer help in deciding the limits of normality, in separating cases of impaired vision due to local ocular causes from those due to deep-seated or widespread disease, and recognizing, from ocular signs, early cases of central nervous lesions. Neuro-ophthalmology, as its hyphenated name suggests, is a synthesis, and its study has been made possible where colleagues of separate disciplines have combined their clinical efforts. It would seem that Dr. F. B. Walsh is as fortunate in his colleagues at the Johns Hopkins University as they are in having him

as their associate professor of ophthalmology. His book is a source of usefulness and of inspiration to neurologists and ophthalmologists alike reminding us all that individualism in medicine, like patriotism in war-time, is not enough.

Eye Surgery. By H. B. STALLARD. 3rd edition, 1958. Pp. 916, 671 figs. Wright, Bristol. (95*s.*)

Stallard's "Eye Surgery" has grown; the third edition is a fat volume 232 pages larger than its predecessor of 1950, 153 illustrations have been deleted and 275 new figures added, and the book has a green jacket instead of a red. The book does British surgical practice credit and has been generally accepted as a standard work; this new edition will certainly consolidate this position. Ophthalmic surgery is essentially an individualistic art; to it the saying *quot homines tot sententiae* certainly applies; and the great value of this volume lies in the fact that it is a record of Stallard's own practice—it makes no pretence at being an exhaustive catalogue of the innumerable operations and modifications of operations to be found in a swollen literature. With his surgical aptitude and experience the author is in a unique position to be a sound guide; and with his extraordinary meticulousness (for each instrument in each operation is detailed, the type of each suture, the composition and timing of each drop instilled into the eye), there is no excuse for the disciple to go wrong. Moreover, the teaching is always sound and reasoned, while the value of superficially attractive innovations such as intra-ocular acrylic lenses or bloodless surgery by hypotensive drugs is sensibly assessed. The book should find a place in every ophthalmological library.

Surgery in World War II (Medical Department, U.S. Army). Ophthalmology and Otolaryngology. Edited by J. B. COATES, Jr., M.C. (1957). Pp. 605. Office of the Surgeon-General, Department of U.S. Army, Washington, D.C.

The story of the war medicine of a campaign is always of interest—of much wider interest than to the soldier. For in modern national war the army, including its medical department, is mainly civilian in habit and spirit and it is interesting to see how, under the strains of a national emergency, the civilian doctor fits into the rigidity of military discipline, and how the regular army adapts itself to an influx which threatens to swamp the routine traditions of peacetime. Neither process is easy; and that it has worked so well in two world wars is a tribute to the universality and brotherhood of medicine. It is also a fascinating story of make-shifts and ingenuity, for as in the British Army, so with the American: no democracy is prepared for a large-scale war; each situation has to be met as it arises; and the lessons, successes, and failures of a previous war have to be relearned.

The present volume concerns itself with ophthalmology and otolaryngology; the first edited by Elliott Randolf of Johns Hopkins, the second by Norton Canfield of Yale University. The ophthalmological section is elaborate and complete. It tells the story of the administrative aspects and clinical policies of the ophthalmological service in the United States (the Zone of the Interior), in the Mediterranean, European, and Pacific theatres, and the arrangements made for the rehabilitation of blinded casualties. Thereafter follows a series of chapters on the clinical management of the more important types of war casualties—intra-ocular foreign bodies, uveitis, retinal detachments, nutritional amblyopia in prison-camps, night-blindness, and visual disturbances following head injuries, with final chapters on plastic surgery and chemotherapy and antibiotic therapy.

It is interesting to realize how long it took for the importance of ophthalmology to be adequately recognized, not only because of the urgency and unique nature of its surgical problems, but because of the operational requirement for the provision of an adequate spectacle service in a national army, for some 15 per cent. of which visual aids were necessary to maintain efficiency at the beginning of the war, a proportion which rose

steadily as the need for man-power led to a progressive reduction in visual standards. The problem appears more serious when it is remembered that in the U.S. Army the average replacement-rate of spectacles for a soldier on active service was 30 per cent. and the average life of a pair of spectacles was 3 to 4 months.

Up to 1944 all matters relating to ophthalmology were handled centrally by surgical consultants; thereafter (as is always ultimately necessary) a special branch of the Office of the Surgeon-General was established. In each theatre the same difficulties were encountered; in each they were finally overcome. The story is a fascinating one and full of human interest. It is a record of which the United States can be proud, not least in the way in which an extraordinarily efficient rehabilitation scheme for the blind was evolved out of nothing.

The Glaucomas. By H. SAUL SUGAR. Second edition, 1957. Pp. 516, 164 figs. Cassell and Co. Ltd., London. (*5 guineas.*)

The first edition of Sugar's book on glaucoma, which appeared in 1951, has been accepted as an able and authoritative exposition of the subject; it is good that a second edition has now appeared. The book follows the general pattern of its predecessor and discusses in considerable detail and with good judgment the physiological, pathological, clinical, and therapeutic problems associated with a raised intra-ocular pressure. The observations of Ashton on the anatomy of the drainage channels have to a considerable extent clarified an important aspect of the subject, and the physiology of the aqueous humour and the problems of its dynamics have been re-written by Everett Kinsey; it is, however, strange to see that the theory of aqueous formation by a redox mechanism is unhesitatingly accepted. Much new material has been incorporated regarding the technique of tonography, but it may be that its clinical implications have been over-simplified. New miotic drugs are amply described and an interesting innovation is a description of Guyton's technique of cyclodialysis. Altogether, this new edition presents an up-to-date and very reasoned picture of a difficult clinical problem written by an author who understands its implication more than most.

NOTES

INSTITUTE OF OPHTHALMOLOGY OF THE AMERICAS

Postgraduate Courses for Specialists

The INSTITUTE OF OPHTHALMOLOGY OF THE AMERICAS of the New York Eye and Ear Infirmary announces a series of Postgraduate Courses for Specialists to be given from November 3, 1958, to January 9, 1959.

Courses will be given in the following subjects: Anomalies of Extra-ocular Muscles, including Ptosis; Aniseikonia; Biomicroscopy; Cataracts; Clinical Bacteriology; Complications of Ophthalmic Surgery; Electrophysiology of the Eye; Glaucoma; Gonioscopy and Tonography; Keratectomy and Keratoplasty; Lacrimal Sac Surgery; Medical Ophthalmoscopy; Near Ultra-Violet Biomicroscopy; Ocular Allergy; Ocular Biochemistry; Ocular Microbiology; Ocular Neuro-Ophthalmology; Ocular Photography; Ocular Radiology; Ocular Trauma; Ophthalmoscopy; Pathology; Perimetry; Plastic Eye Surgery; Pleoptics and Macular Function Testing; Psychosomatic Factors in Ophthalmology; Radio-Isotopes in Ophthalmology; Refraction; and Retinal Detachment.

Further information regarding the Courses may be obtained by writing to Mrs. Tamar Weber, Registrar, Institute of Ophthalmology of the Americas of the New York Eye and Ear Infirmary, 218 Second Avenue, New York 3, New York.

International Course in Ophthalmology, Barcelona

A second International Course in Ophthalmology will be held at the Barraquer Institute, Barcelona, from September 16 to October 1, 1958.

There is a competition for scientific papers on ophthalmological subjects open to any medical practitioner under 40 years of age, of any country, and written in any language. Three prizes of 5,000, 3,000, and 1,000 pesetas will be awarded as well as several prizes of 500 pesetas. Papers must be submitted by December 31, 1958. Particulars may be had from Dr. Joaquín Rutllán, Instituto Barraquer, Laforja 88, Barcelona, Spain.

Irish Ophthalmological Society

The annual meeting of the Irish Ophthalmological Society was held in Dublin on 1, 2, and 3 May, 1958. The Montgomery Lecture was given by Prof. Hagedoorn (*Amsterdam*) in Trinity College, Dublin.

Pan-American Congress of Ophthalmology

The VI Pan-American Congress will be held in Caracas during January–February, 1960. Further details may be had from:

Dr. Edward Grom, Executive Committee, Appartado 5666, Chacao, Caracas, Venezuela.

Ophthalmological Society of Pakistan

This Society came into existence on December 19, 1957, when the following office-bearers were elected:

President: Lt.-General W. A. Burki
Vice-Presidents: Prof. Ramzan Ali Syed; Prof. Rifat Ullah
Secretary: Dr. Raja Mumtaz
Treasurer: Dr. M. S. Faridi

Study Group for Regional Ophthalmology

Ophthalmologists interested in the relationship of geographic, environmental, and genetic factors to eye diseases are invited to attend a meeting at 11 a.m. on September 11, 1958, at the Palais de Beaux Arts, Room: La Rotonde, 23 Rue Ravenstein, Brussels.

Southern Ophthalmological Society

Change of Date

The date of the meeting of the Society arranged for Saturday, September 27, 1958, at the Sussex Eye Hospital, Brighton, has been changed to Saturday, October 4, 1958.

Brit. J. Ophthal. (1958) **42**, 512.

OBITUARY

Otto Barkan

Surgeons all over the world will regret the death of Otto Barkan on April 26, 1958, at the age of 71. He was the son of Dr. Adolph Barkan, a Hungarian who was one of the original staff in the Eye, Ear, Nose and Throat Department of Stanford University School of Medicine. The son followed in the footsteps of his father, and in his medical education he travelled widely. He studied at Trinity College, Oxford, obtained the B.A. degree in 1909, graduated in medicine in Munich in 1914, and later served as a house-physician in St. Mary's Hospital, London, and became a member of the Royal College of Surgeons of England. His essential eye training was received at Munich and Vienna. Returning to San Francisco in 1920, he became a member of the Faculty at Stanford University where he worked for the remainder of his professional life, practising ophthalmology in San Francisco. He gained a world-wide reputation by his studies on glaucoma and was a pioneer in stressing the importance of the width of the angle of the anterior chamber in the differential diagnosis between closed-angle and simple glaucoma. He will always be remembered for the introduction of the operation of goniotomy (trabeculotomy) in the congenital form of this disease, a technique which has revolutionized the prognosis of this intractable condition in many cases.

In his life-time, Otto Barkan's achievements were duly recognized and his professional interests and contacts were world-wide; apart from being a member of many American Societies, he was a member of the British, French, and Belgian Ophthalmological Societies. He was also a keen sportsman; he rowed for Oxford University and was an ardent skier and Alpinist. A delightful companion, a courteous host and an enthusiastic colleague, he will be much missed by his many friends all over the world.

Henry Kirkpatrick

Ophthalmologists particularly in Great Britain and in India will be sad to hear of the death of Lt.-Col. H. Kirkpatrick, who spent most of his life in the Indian Medical Service. He died peacefully at his home in Hampshire on May 11, 1958, at the age of 86.

Educated in Dublin, where he qualified in 1894, he did his postgraduate work in St. Mark's Eye and Ear Hospital in that city under the instruction of Swanzy and Werner. In 1898 he joined the Indian Medical Service and was appointed to the staff of the General Hospital, Madras, where he acted as physician and professor of pathology. There he came under the influence of Col. R. H. Elliott, whom he succeeded in 1914 as Superintendent of the Government Ophthalmic Hospital and Professor of Ophthalmology in Madras. While in this office he supervised the construction of the Elliot School, a large teaching institution, and spent much of his time in improving the facilities for postgraduate training particularly in pathology. Leaving Madras in 1920, he started consulting practice in London, again initially in association with Elliot, becoming ophthalmic surgeon to the Hospital for Tropical Diseases, and lecturer in ophthalmology at the London School of Hygiene and Tropical Medicine. In 1937 he gave up consulting practice and retired to live in the country in Hampshire.

Kirkpatrick was an excellent ophthalmologist, an accomplished surgeon, and a conscientious teacher. His greatest achievement was the advancement of ophthalmology in South India where many of his old pupils will regret his death. He made a considerable number of contributions to ophthalmic literature, published two small volumes—*Cataract and its Treatment* (1921) and *Diseases of the Eye* (1936)—and collaborated with Elliot in the production of his standard textbook—*Tropical Ophthalmology* (1920). A kindly, unassuming, and courteous man, he was beloved by all who came in contact with him. He is survived by a wife and two daughters.